TEAM 1: BIOVOLT

The Research and Development of a Microbial Fuel Cell

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Executive Summary

Microbial fuel cells (MFCs) are an emergent technology that offers a novel approach to small scale electrical power generation that could be useful for recharging batteries for use in cameras, medical devices, or other battery-operated devices in areas without access to the capital needed for more traditional means of electrical generation. BioVolt designed, optimized, and constructed a prototype MFC that built upon existing research, making an electricity generating device that is more cost effective.

BioVolt's final prototype consists of a cell casing made of polyvinyl chloride (PVC), an Ultrex proton exchange membrane, a set of eight plain graphite electrodes in the anode where the biofilm of bacteria is deposited, a set of four graphite electrodes in the cathode where the reduction of oxygen to water takes place, and a filter / pump system attached to a media storage chamber for the semi-continuous injection of clean media into the anode. BioVolt also designed a simplified, inexpensive media solution composed of vinegar, baking soda, and a salt solution.

The functional prototype produces 0.5μW at 0.6V, provides ten days of use before one third of the media must be refreshed, is portable, operates on a feed obtainable by the user, and proves the validity of the concept of using an MFC for inexpensive, small scale electrical production. The power output of the cell can be improved 1,000 to 10,000 times that of this prototype by the addition of a platinum catalyst to the cathode electrodes (approximately 50mg of platinum are needed) and 10 to 100 times by using a bacterial cocktail in the anode chamber. Further research is needed with these cases before implementation; however, this research is beyond the scope of BioVolt's project.

1 Introduction

Alternative energy is a highly discussed subject in today's society and is also an area of extensive research. As part of the Bachelor's of Engineering degree at Calvin College, senior engineering students form teams and spend both semesters of their senior year attending a class called Senior Design (ENGR 339-340). The intent of this series of classes is to work as a group and tackle a project of the teams choosing. Team 1: BioVolt, comprised of four chemical engineering students, chose to take on a project that covers alternative energy, and in particular, the production of electricity using a microbial fuel cell (MFC).

Certain bacterial species, in the course of their normal cycles of metabolism and respiration, have the ability to transport electrons that are produced during these processes to materials outside of the cell membrane. While this act of transporting electrons outside of the cell membrane is specific to only a few bacterial species, recent studies have shown that by providing the correct strain of bacteria with optimal growth conditions and an electron acceptor, an electrical current can be generated. Microbial fuel cells, sometimes also referred to as "bio-batteries," take advantage of this process and have become an area of intense research in the field of alternative energy.

BioVolt set out to replicate and build on the research in the area of MFCs by creating a prototype which would demonstrate MFC's potential for low maintenance operation, use a bacterial feed comprised of ingredients easily attainable by the customer, and provide electricity over an extended time period. Since MFC power output is relatively low, BioVolt's intended customers are people living and/or working in rural areas that do not have readily available access to conventional electrical grids. People living in these areas would benefit from a very low cost device that could deliver enough electricity to power low voltage lights, and/or recharge batteries for medical or other devices.

2 Problem Specification

2.1 Project Scope

The scope of this project is to take ideas being generated in current research on microbial fuel cells and apply them to produce a fully functional prototype that could potentially be used commercially. This project focuses on engineering design and optimization of the fuel cells functional unit, while meeting specified objectives. It is assumed that any power regulation necessary to accomplish a specific task (i.e. charging a battery or powering a low power device) can be purchased separately and simply attached to the functional unit.

2.2 Project Objectives

2.2.1 Sustainability

This project is intended to demonstrate the creation of an environmentally-friendly form of energy production. To accomplish this objective, the design must produce a minimal amount of waste during its operation and disposal.

2.2.2 Size

In order to fulfill the design considerations for the intended customers, the working prototype must be transportable from one location to another. Therefore, the size and weight of the prototype must promote reasonable portability.

2.2.3 Feed

The feed, or media, used under research conditions for microbial fuel cells is typically a complicated solution of laboratory chemicals in precise concentrations. In order to produce a prototype that promotes use in the intended market, BioVolt must design a feed/media formulation that is comparably effective as the laboratory media, is inexpensive, and can be constructed from readily available components, using common measuring utencils.

2.2.4 Lifetime

The design of a final prototype must take into consideration means by which to maximize the functional lifetime of the MFC. The prototype MFC should be able to last for one year with minimal user intervention or maintenance.

2.2.5 Power Output

The goal set forth by this project is to build upon existing research and improve upon the accomplishments published to date. Since many other objectives have an effect on power output, the project goal is to produce at least a comparable power output to the published data. According to Rabaey and Verstraete in *Microbial fuel cells: Novel biotechnology for energy generation*, a power output of 10-20 mW/m^2 of electrode surface area is obtainable for a prototype.

3 Project Management

3.1 Work Breakdown Structure

The project work has been distributed among the four chemical engineers in the team. Each individual made a significant contribution to the project and tasks were divided so each team member's involvement was essential in the successful completion of the project. All were responsible for completing their individual parts on time and for helping the other team members with their assignments when necessary.

- Lindsay Arnold was responsible for the research involved in the project as well as assuring all tasks were completed on time. She was in charge of the biological logistics of the MFC, and learned the variety of biological protocols and procedures used for growing the bacteria needed for the cell. She designed and performed media and kinetics experiments and analysis, and optimized media for bacterial growth and cost efficiency.
- Jeff Christians was responsible for the electrical concepts of the project, which included selecting the anodes and cathodes necessary, as well as all internal design decisions for the final prototype. He was also responsible for prototype conceptualization and design, utilizing his expertise in Inventor throughout the

designing process. Jeff also participated in final design assembly. He was also in charge of visual deliverables, such as the user manual and various process flow diagrams.

- Diane Esquivel was responsible for set up of the appropriate feedstock for the MFC. She also learned the variety of biological protocols and procedures used for growing the bacteria needed for the cell and aided in the bacterial experiments. Diane was responsible for keeping track of the cost of used and needed materials, as well as the overall budget of the project and the potential business plan marketing analysis.
- Andrew Huizenga was responsible for the ordering and acquisition of all materials needed for the final prototype as well as all research prototypes used in the experimentation process. He was the primary contact with Membranes International and made internal design decisions and selections. Andrew was in charge of prototype construction, for both the final prototype and the research prototypes.

3.2 Schedule

Because this project was quite expansive, Gantt charts were used to properly budget the necessary time to complete the various tasks. Through careful time management, the project progressed in an efficient and timely manner. The Gantt charts are broken down by semester, and display major tasks relevant to the design and construction process. Weekly meetings were utilized to track progress, discuss ideas, and solve issues associated with the design process.

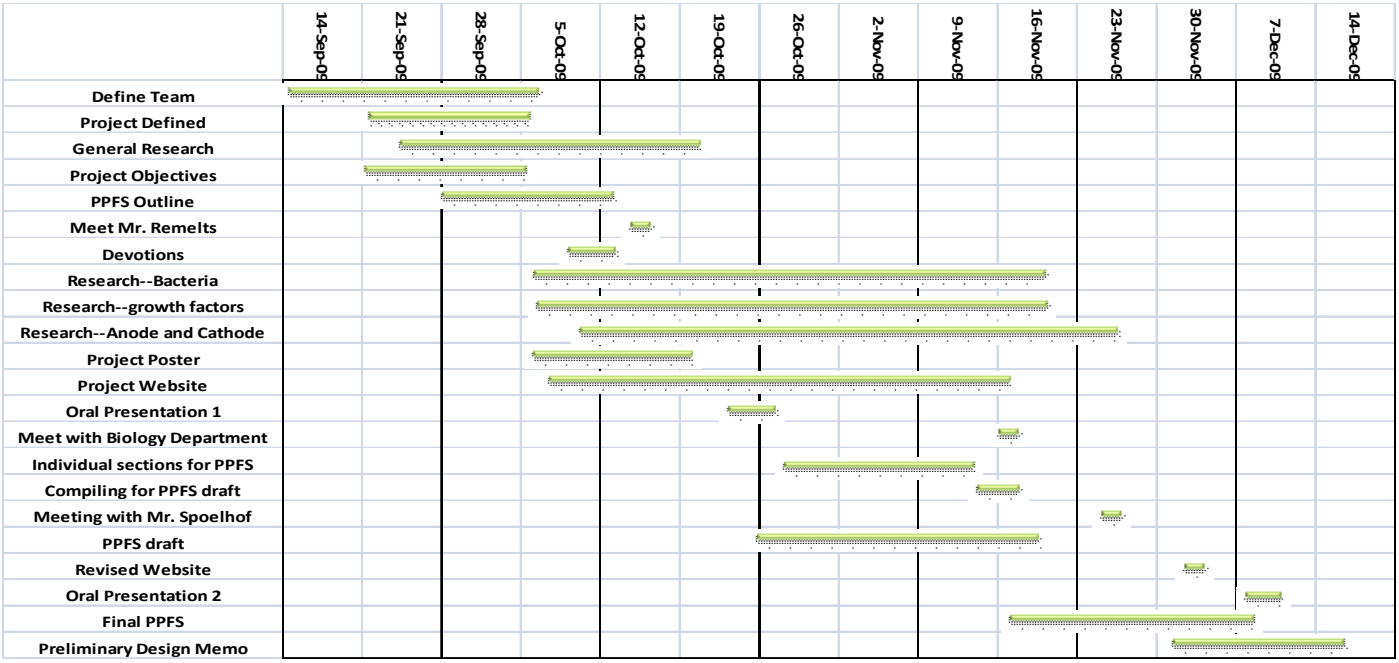


Figure 1 - Fall semester Gantt chart

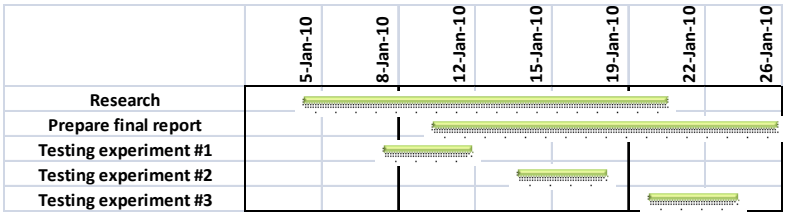


Figure 2 - Interim Gantt chart

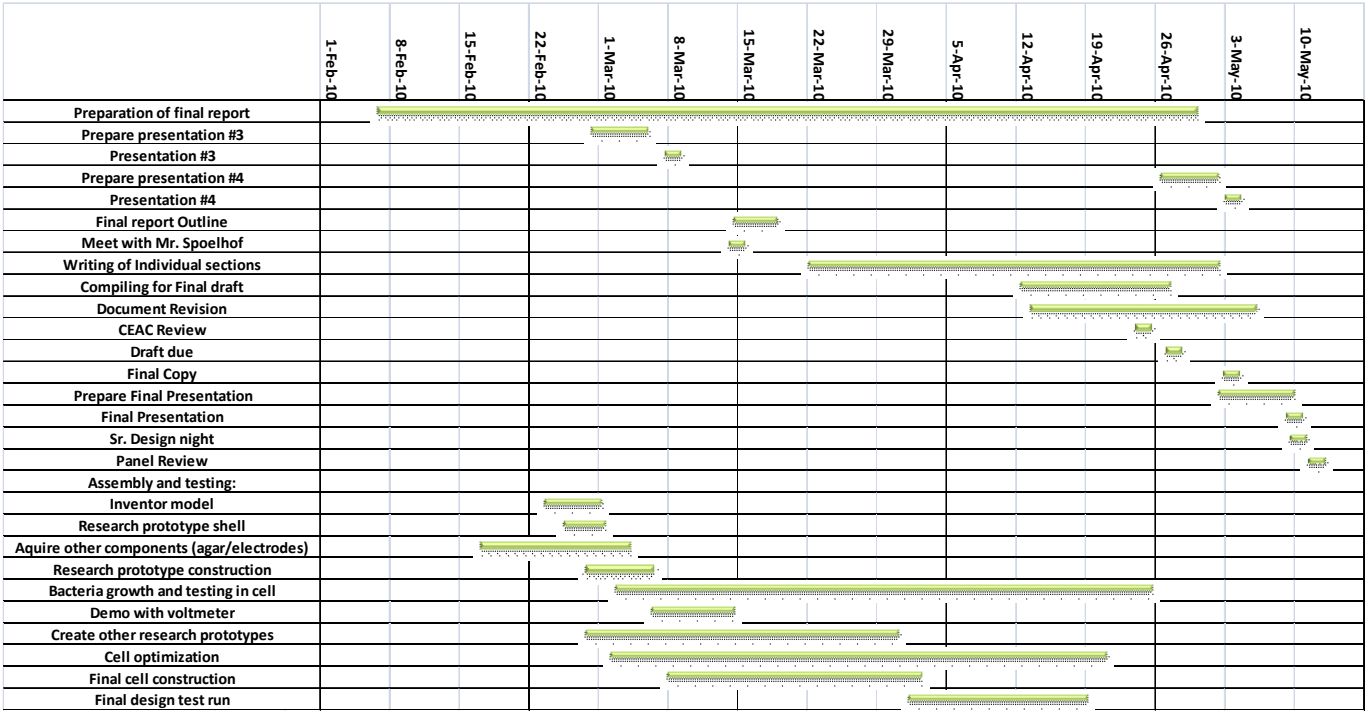


Figure 3 - Spring semester Gantt chart

4 Project Budget

4.1 Prototype Budget

This project was funded by the Calvin College Engineering Department. An initial amount of $300 was allotted to BioVolt as a suggested project budget. After calculating a rough estimate of the project budget, including design materials, bacteria cultures, and bacterial growth nutrients, it was projected that the materials needed for construction would total $295. This budget was revised based on the cost of purchasing the *Geobacter sulfurreducens* and a new budget of $500 was proposed. Critical decisions were made before purchasing any component of the MFC prototype, and the budget was updated following each purchase. The updated budget was reviewed each week during weekly meetings and important issues concerning the budget were handled.

Table 1 is a list of final expenses relating to the completion of the project. A few items, specifically the Ultrex membrane and the media ingredients, were donated to BioVolt, and therefore have a cost of $0. Appropriate acknowledgements are given to these donors on page 33. Table 2 is a list of expenses pertaining to the project if all aspects of the design were purchased.

Table 1 - Actual Prototype Budget

Part	Cost	Qty	Where acquired
G. sulfurreducens	$195	1	ATCC
G. sulfurreducens shipping	$109	1	ATCC
Wolfe's Mineral Solution	$50	1	ATCC
Wolfe's Vitamin Solution	$50	1	ATCC
Bacteria Medium Solution	$0	---	Calvin Biology Dept
Cell Electrodes	$23	4	HobbyLinc.com
Pump	$12	1	
Construction Material for research prototypes	$20		Lowes
Construction Materials for final cell	$23		Lowes
Ultrex Proton Exchange Membrane	$0	1	Membranesinternational.com
Total	**$482**		

Table 2 - Full Cost (Theoretical) Prototype Budget

Part	Cost	Qty	Where acquired
G. sulfurreducens	$195	1	ATCC
G. sulfurreducens shipping	$109	1	ATCC
Wolfe's Mineral Solution	$50	1	ATCC
Wolfe's Vitamin Solution	$50	1	ATCC
Bacteria Medium Solution and buffer	$36	1 of each ingredient	Calvin Biology Dept/Aldi
Cell Electrodes	$23	4	HobbyLinc.com
Pump	$12	1	
Construction Material for research prototypes	$20		Lowes
Construction Materials for final cell	$23		Lowes
Ultrex Proton Exchange Membrane	$20	1	Membranesinternational.com
Total	**$538**		

4.2 Production Budget – Economic Analysis

If this prototype were produced on a commercial scale, the unit cost would be dramatically reduced from the prototype cost. Here, the production cell unit cost is divided into three sections: the biological costs, the materials costs, and the yearly media costs. The total per unit material cost based upon a ten thousand unit production totals a conservative estimate of $7.71.

Table 3 - Estimated Production Unit Materials Cost

Part	Cost
Biological Costs	$0.75
Cell Electrodes	$0.96
Pump	$0.91
Casing Material	$1.30
Ultrex Proton Exchange Membrane	$0.47
Media Costs	$3.00
0.22 Micron Filter	$0.06
Electrical Components	$0.26
Total	**$7.71**

4.2.1 Biological Costs

On a commercial scale, the bacterial cost is an initial, onetime cost, plus the cost of growing and farming the bacteria cultures. Once there is an initial source of bacteria, the bacteria multiply if given the proper habitat and nutrition, so the only cost associated with the biological aspect of the design is the cost of maintaining the bacterial growth, and farming cultures of the bacteria for use within a cell. Costs associated with farming the bacteria, not including facilities and operating costs, are only associated with the cost of the nutrient/feed material. The cost of the media is minimal, chemicals can be purchased in bulk and the concentration of each chemical in the media is low. It is estimated that for a production of ten thousand units, a conservative cost estimate of the biological components per unit is 75¢.

4.2.2 Construction Materials

Commercially, more cost effective materials will be used to construct the MFCs. Instead of PVC pipe, the cell casing will be constructed out of blow molded HDPE at an estimated production cost of $1.30 per complete unit.

The prototype contains a round, roughly nine square inch membrane. By altering the configuration of the membrane used for commercial production to a 3x3 square, the same surface area can be achieved with almost no scrap per membrane. Based on a ten thousand unit production, purchasing the membrane in bulk quantities results in a per unit cost of 47¢.

The feed system consists of a primer bulb, one foot of Tygon© tubing, a silicon check valve, and a 0.22 micron filter. Assuming the primer bulb is purchased and not manufactured in house, the total cost of the pump feed system for a ten thousand unit basis is 91¢ per unit plus 6¢ per unit for the filters.

Based upon an eight plus four electrode setup, each being four inches long, the total cost per unit for the electrodes is approximately 96¢. The cost per unit for all of the electrical components (wiring, terminals, terminal epoxy, etc) totals 28¢.

Thus, commercially producing ten thousand units would result in a materials cost of $4.71 per unit.

4.2.3 Operating Costs

To produce ten thousand units, there would need to be facilities large enough to handle the microbiological growth requirements. Machines would have to be purchased to mold the casings for each unit, and either machines or a labor force would have to be in place to assemble each unit. From a conservative estimate, about $3.00 per unit will be added to cover operating costs.

One hurdle that needs to be investigated and overcome before BioVolt's MFC could become commercialized, is the aspect of shipping the final kit, including bacteria, to the end customer. Shipping microbiological cultures is a tightly regulated process, both legally and logistically. Most cultures need to be shipped cryogenically, so as to reduce the chance of cell death by the time the package is delivered. This is extremely costly (reference Table 1). Shipping across borders also poses legal issues with customs. Therefore, BioVolt initially would plan to base productions in a location that is local and central to their targeted customers.

5 Design

5.1 Design Considerations and Criteria

5.1.1 Projected Customers

5.1.1.1 Profile

Because of the low initial cost, low operating costs, and low power output associated with an MFC, BioVolt's main market is people who live or work in areas without access to conventional means of power production, but still have need of small amounts of electrical power for applications such as low voltage lighting, recharging batteries for medical devices. For example, BioVolt is targeting rural missions and rural health providers; hoping to provide a much needed source of energy to power low voltage lights, recharge battery operated medical equipment, cameras, emergency cellular phones, etc.

5.1.1.2 Resources

Limited resources are available for the construction and maintenance of the MFC in the area of projected use. Since the projected use of the MFC is in rural and undeveloped areas, designing a prototype while maintaining a low cost will result in a much broader impact. The materials for building MFCs should be readily available or easily obtained, and the feed to the cell must be inexpensive and simple to construct.

5.1.2 Design Norms

5.1.2.1 Stewardship

This project is based on BioVolt's commitment to use natural resources to create sustainable, earth friendly energy for use in areas where energy is not readily or economically available. In today's growing energy crisis, carbon neutral, sustainable and cost effective energy production is highly prized. Development of alternatives like MFCs is the key to preserving the environment and weaning this technological age off burning coal. Similarly, it is the duty of Christians to use the resources given by God in a way that is pleasing, efficient, and resourceful. Wastefulness and irresponsibility in daily lifestyles are not feasible for the long term or a portrayal of good stewardship. By harnessing a naturally occurring process and using it to produce sustainable energy, Team BioVolt demonstrates a renewable source of electricity. Additionally, good stewardship encourages the design to be as cost effective as possible, because being a good steward also pertains to the efficient use of resources, such as capital. Creating a cost effective MFC also encourages broader application, and allows this sustainable technology to become more widely deployed.

5.1.2.2 Trust

Gaining the trust of any customer who would purchase and operate a microbial fuel cell is an important design norm that impacted the prototype design of this project. Having a reliable power source is crucial especially when it is the only source of power available, as the MFC would be for nearly all of the projected customers. Unexpected failures could result in lost time, expensive repairs, frustration by the consumer, and, if being used to charge medical equipment, possible physical harm. If the MFC is not dependable, potential clients will not invest in the technology, rendering the MFC

ineffective in fulfilling the customer's energy needs. It is also never within a Christian perspective to produce an unreliable or untrustworthy product. A Christian commitment encourages a quality result.

5.1.2.3 Design Transparency

The design process of this microbial fuel cell was carefully documented. This documentation makes the expressed results reproducible from the documented research and experiments, so further testing and optimization could build upon this research. Aside from replication, this design needed to be transparent so that users can understand the functionality of the product and are able to maintain and use the product to its full potential. Transparency through organization ties in with the Christian perspective on trust; people will trust a technology that they understand. The general public should be able to easily understand the operation and function of this form of energy production. This level of understanding will encourage public awareness and stimulate interest in the design and ultimately, the technology itself.

Furthermore, if the MFC is to be in the intended locations, it must have simple, intuitive operating procedures that require little or no biological expertise. This means the design should be basic enough so troubleshooting by the general public is possible. If this is not possible, MFCs will be an alternative energy only available to the scientifically educated public, not the desired users.

5.1.2.4 Integrity

Integrity has been a cornerstone for BioVolt over the course of the design process. The gathered research and assistance of others have been given due credit. In the lab, multiple experiments were performed to ensure reproducibility and no lab work or record has been falsified. If the design procedure had not progressed with integrity, the final product could have been faulty, harmful, or misleading. All challenges and failures have been addressed in the report so as not to give the reader false impressions of success or incorrect information.

5.1.2.5 Cultural Appropriateness

Cultural appropriateness is crucial for the successful implementation of the MFC. If the MFC does not meet the needs of the customer, then the technology will not be adopted. This means that part of the design process of the MFC focused on adapting the technology to the current culture and making as few additional demands from the users as possible. This applies to the materials chosen to produce this MFC as well as the design as a whole. If the components of the cell are not readily available or the ingredients of the media are expensive and uncommon, upkeep, use, and implementation of the MFC becomes non-feasible. Only by creating a culturally appropriate product can BioVolt begin to address the energy needs in developing areas.

5.1.3 Environment, Safety and Health

5.1.3.1 Environment

A key aspect of the MFC design is the environmentally friendly nature of the energy source. The main product of bacterial digestion is carbon dioxide, and the waste from the anode chamber is composed primarily of unused nutrient feed, which contains acetate, table salt, baking soda, ammonium chloride, and sodium phosphate in water. These components are not harmful to the environment, and are suitable for watering plants.

The MFC is designed to be refilled and reused, but if a non-functional MFC is to be disposed of, it is constructed out of 100% recyclable materials.

Bacterial disposal should be handled with care. The bacteria species is non-toxic; however, improper disposal could result in damaging environmental effects. Inoculated media should not be disposed of in any body of water, as the microorganisms remain viable in anaerobic aqueous solutions. An effective disposal technique involves exposure of the bacteria to oxygen and UV light (sunlight) for one week, or boiling for five minutes. These methods effectively neutralize the bacteria allowing for safe disposal of the media.

5.1.3.2 Safety

The MFC is inherently safe. Care should be taken when handling electrical devices. Proper instructions for connecting and disconnecting an electrical device to the MFC should be followed.

5.1.3.3 Health

The ingredients in the media are mainly kitchen appropriate materials: water, baking soda, vinegar, and table salt. If consumed in large quantities, patient may experience some discomfort, but these materials are safe for handling and even mild consumption. Two components necessary in the media that are not substitutable as purchasable kitchen ingredients are ammonium chloride and monobasic monohydrate sodium phosphate. Neither of the two components pose serious health risks, however ammonium chloride is a mild irritant and slightly acidic (pH = 5.5) when dissolved in water. The MSDS for ammonium chloride and monobasic monohydrate sodium phosphate are provided in Appendices E and F.

5.2 Internal Design Alternatives and Analysis

5.2.1 Overview

The bacterial species used within the final prototype is *Geobacter sulfurreducens*. Through a review of the literature, BioVolt determined that this species of bacteria is a strong candidate for use in an MFC because it can be grown in a simple nutrient media composed primarily of acetate. The media used in the final prototype is a simplified variation of the lab grade media supplied for bacterial growth. This media is composed of water, baking soda, vinegar, table salt, ammonium chloride, and sodium phosphate. Much of this media is constructed from common household items, however, small quantities of the lab-grade chemicals, ammonium chloride and sodium phosphate, are also needed.

5.2.2 Bacteria Culturing

Through research into the performance of many different bacterial species shown in previous research, the bacterial selection was narrowed down to three species, *Geobacter sulfurreducens* (GSR), *Geobacter metallireducens* (GMR), and *Rhodoferax ferrireducens*

(RFR). These species were researched and evaluated based on five criteria: price, power production, accessibility, caring, and the required media. Table 4 summarizes how the three different species were compared and evaluated.

Table 4 - Bacterial Species Decision Table

Bacterial Species Decision Table				
	Weight	GSR	GMR	RFR
Price	20	7	7	7
Power	35	8	7	5
Accessibility	5	8	9	6
Caring	15	8	8	8
Feed	25	6	6	4
Total		**730**	700	565

The most influential factor of the bacterial species selection was the power achievable by the given microbe. In the literature, certain species outperformed others on a regular basis. Specifically, the *Geobacter* strains yielded higher power output per electrode surface area than did the *Rhodoferax* (Rabeay 294). With power production capability being such an important design specification, it is weighted heavily in Table 4. As shown in the decision matrix, *Geobacter sulfurreducens* proved to be the best overall choice of bacteria for BioVolt's needs.

5.2.3 Media/ Habitat

To reduce the cost associated with the microbial habitat, BioVolt designed a feed solution for the final cell which was comprised of vinegar, baking soda, non-iodized table salt, ammonium chloride, and sodium phosphate, as shown in Table 5. This solution was used to ensure that all of the materials in the feed were low cost and easily accessible, following the design norm of stewardship and cultural appropriateness.

Table 5 - Media Recipe

Medium Component	Amount (g/L)
Sodium Chloride	1.5
Sodium Phosphate	0.6
Morton salt	0.1
Baking soda	2.5
Vinegar	0.82

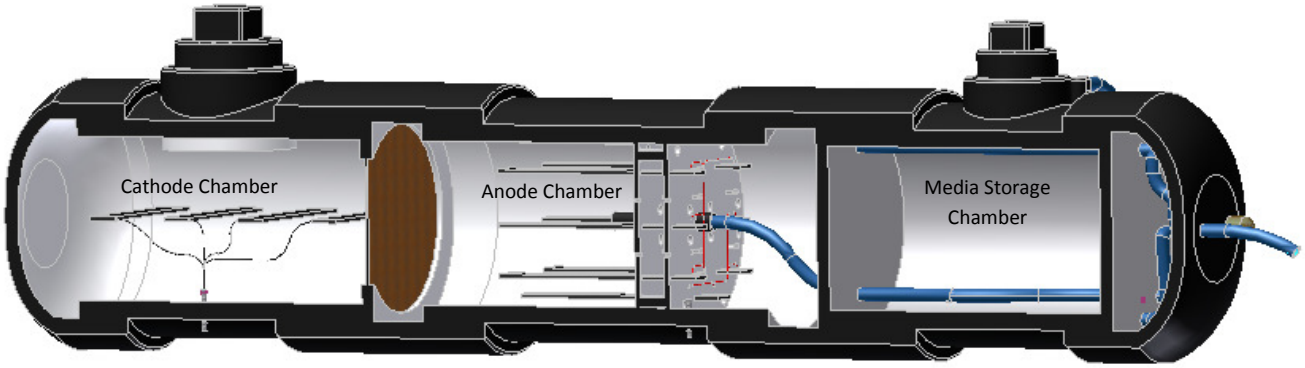
The pH of the solution for *Geobacter sulfurreducens* must remain relatively neutral: 6.8-7.0 pH. The medium created for these organisms must also remain essentially sterile. Maintaining a sterile media reduces the risk of contamination with other competing microbes. In addition, the bacteria must not be exposed to large amounts of UV light or oxygen. To achieve the best possible operation, the final prototype must account for all of these concerns.

Initially the bacteria were grown with a few other components, such as sodium fumarate and Wolfe's vitamin and mineral solutions; however, through a series of experiments (Appendix B), it was found that the bacteria did not require these components to grow, so they have been omitted for the final media. The initial ATCC media recipe can be found in Appendix D.

5.3 External Design Alternatives and Analysis

5.3.1 Overview

Figure 2 - Final Prototype Design



The final prototype includes a three-chambered design. The prototype is designed to be fully enclosed to minimize the risk of anode chamber contamination and protect the bacteria from exposure to sunlight and oxygen. The design incorporates threaded caps that allow for the prototype to be transported easily without creating a spill hazard.

Design Component Descriptions

Media Storage Chamber: The Media Storage Chamber is where fresh media is stored for later use within the cell. The volume of the Media Storage Chamber is adequate to provide full media replacement of the Anode Chamber and under the suggested operating procedures, can last up to two months.

Anode Chamber: The Anode Chamber is completely sealed with the exception of a service port to drain and/or fill the chamber initially. The Anode Chamber is filled completely with the nutrient media and contains the microorganisms as well as the electrodes upon which the organisms grow.

Cathode Chamber: The Cathode Chamber is open to the atmosphere under normal operating conditions, although a threaded cap is provided to prevent leakage during transportation of the cell. The electrodes are submerged in an aqueous buffer solution, providing ideal conditions for the reduction reaction to occur with oxygen, completing the electrical circuit.

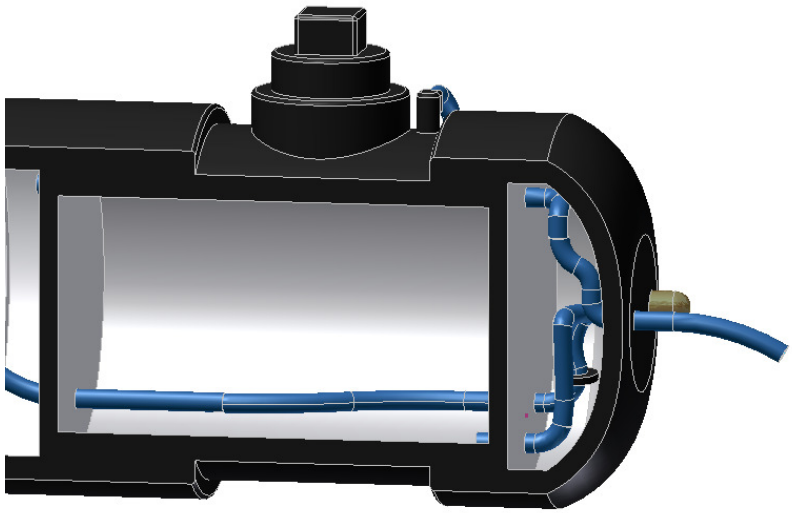


Figure 3 - Injection apparatus close-up view

Media Injector: The media injector is a primer bulb that takes media stored in the Media Storage Chamber and pumps it through the Pre-Injection Filter and into the sealed Anode Chamber.

Media Outlet: The Media Outlet is connected to the Anode Chamber using a one-way valve. When the pressure in the Anode Chamber is increased by the injection of new media, spent media is ejected through tubing to the Media Outlet located on the end cap.

Pre-Injection Filter: This is a replaceable filter that is rated for 0.22 microns, used to filter the media as it is pumped from the Media Storage Chamber to the Anode Chamber. The filter resides behind the Media Storage Chamber end cap, which is removable so as to facilitate filter replacements.

Level Indicator: The level indicator is a tube connecting the top of the Media Storage Chamber to the bottom of the chamber. This allows a visual indication of how full the Storage Chamber is without having to remove the fill cap.

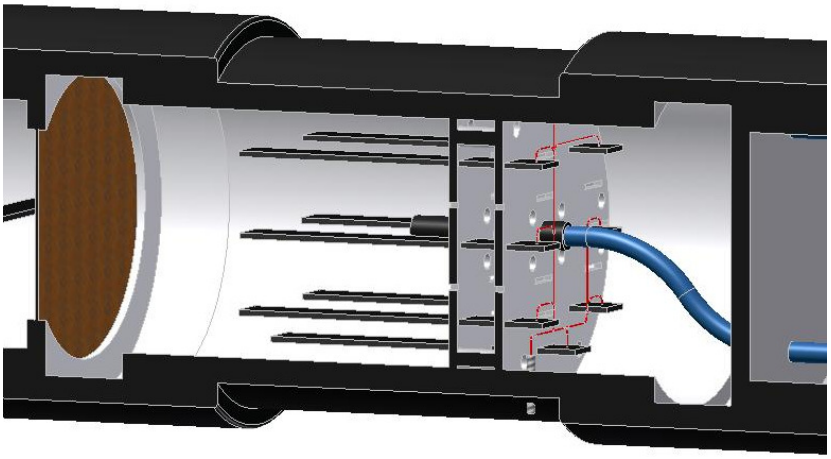


Figure 4 - Anode chamber close-up view

Anode Electrodes: Eight electrodes composed of graphite are wired in parallel to a single terminal in the Anode Chamber. The microorganisms form a biofilm upon the electrodes, providing the electrons needed to produce electricity.

Proton Exchange Membrane: The Proton Exchange Membrane is made from Ultrex (CMI-7000 Cation Exchange Membrane) and separates the Anode Chamber from the Cathode Chamber. The membrane is the key to the functionality of the cell since it is the membrane that forces the electrons to run through external wiring (load) in order to get to the Cathode Chamber.

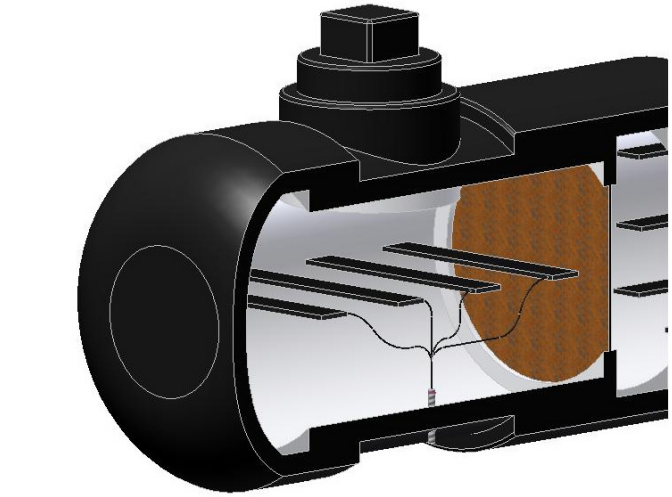


Figure 5 - Cathode chamber close-up view

Cathode Electrodes: There are four cathode electrodes composed of graphite that are wired in parallel and connected to a single cathode terminal. The reduction of oxygen occurs on the surface of these electrodes, completing the electrical circuit between the Anode and Cathode Chambers.

5.3.2 Casing Material

The prototype casing material was selected on the basis of several key design factors:

- Availability: In keeping the design norm of stewardship, it is important that the material be widely available.
- Cost: To conserve the resources of the customer, it is important that the casing be as inexpensive as possible.
- Opaquacity: The material must be opaque in order to block UV light from reaching the bacteria.
- Durability: The material must be durable in order to handle the possibility of dropping, the potentially harsh conditions where the MFC is to be deployed, and the constant exposure to the bacteria and feed solution.
- Workability: The material must be simple to work with and require only limited labor and assembly.

Based on these criteria, two materials were investigated for possible use. Both steel and PVC are widely available, so both scored very high in this category. This was a highly rated decision factor because of BioVolt's desire to market this device in rural areas without access to many specialty materials. These two materials also are very inexpensive, which is in keeping with the design norm of stewardship; however, when comparing the two, PVC is significantly less expensive than steel.

Because both materials block 100% of incoming UV light, both were valid options for construction the cell casing. The durability of PVC is higher since the cell would constantly be exposed to the bacterial feed solution and the phosphate buffer in the anode and cathode respectively. This constant exposure to a salt solution would cause the steel to rust at an increased rate unless the cell was made out of an expensive metal such as copper or a high grade stainless steel, and, in following the design norm of stewardship, the extra cost associated with either of these options was deemed to be unacceptable.

Because both materials are easy to work with, opaque, and widely available, PVC was selected due to its increased durability and relative expense as shown in Table 6.

The following decision matrix shows the two main materials, steel and PVC, that were considered for the construction of the cell case. Both materials were given a score from 0 to 10 points in four categories each weighted respectively to how important that aspect is to the design. The scores are multiplied by the category weights to achieve a final score for each material in question. The material with the highest combined final score proves to be the best material based upon the design categories.

Table 6 - Cell Casing Decision Matrix

Category	Category Weight	Steel	Steel Subscore	PVC	PVC Subscore
Availability	20%	10	2	9	1.8
Inexpensive	25%	7	1.75	9	2.25
Opaquacity	10%	10	1	10	1
Durability	35%	7	2.45	9	3.15
Workability	10%	9	0.9	9	0.9
Final Score	100%		**8.1**		**9.1**

5.3.3 Electrodes

Previous literature examples of MFCs using *Geobacter sulfurreducens* have used several different materials for the anode and cathode electrodes (Dumas et al. 2495). The most widely used and inexpensive materials were stainless steel and various forms of graphite. Both stainless steel and graphite were shown to produce comparable results. Different types of graphite were evaluated, such as graphite foam, woven graphite, plain graphite rods, and carbon paper (Rabaey 294). Platinum plated graphite electrodes proved to perform better than non-platinum plated graphite or stainless steel electrodes, but are quite expensive for prototyping. Based upon cost alone, electrodes for both chambers made of plain graphite were chosen as the optimal material for the prototype design.

5.3.4 Feed System

Geobacter sulfurreducens is both oxygen and UV light sensitive, and must be isolated from outside bacterial species. Because of this, a simple feed system was designed which required no laboratory skills or equipment and does not expose the bacteria in the anode chamber to large amounts of oxygen, UV light, or foreign bacteria. Several different possible designs were evaluated including injection of the bacteria via a syringe, a pump system, and creating a physical barrier via an immiscible liquid layer.

A pump system was chosen for the final prototype based on evaluation of each of the possible options against the following criteria:

- Isolation from Oxygen: The feed system must isolate the bacteria from oxygen acceptably to be a valid option for implementation.
- Isolation from Outside Bacteria: To produce and MFC which can operate for extended periods of time all competing bacterial species must be eradicated.
- Ease of Use: The system should be easy to use and maintain so the MFC is a hassle-free means of power production.
- Intuitive Design: In keeping with the design norm of transparency, the feed system design should be clear and intuitive so the consumer is able to understand, use, and maintain it.

- Reliability: The feed system must be reliable so that its performance does not negatively interfere with the overall performance of the MFC.
- Cost: To conserve the resources of the customer, it is important that the feed system be as inexpensive as possible.

The three feed system options, a syringe injection, a pump system, and using an immiscible liquid layer, all provide adequate isolation from atmospheric oxygen. The syringe feed system and the immiscible liquid would protect the bacteria from oxygen better than the pump system. However, the small exposure to oxygen which each of these options would give is negligible.

To effectively protect the MFC against outside bacterial species, all three of these design choices must be slightly modified. One effective way of keeping out bacteria would be filtering all incoming feed solutions. This would be simple to do, require inexpensive and widely available filters, and be easy to integrate into all three of the design options. In this way, all three of the potential options rank equally well as they all use the same basic method for filtering. It would, however, be easier and less prone to error to integrate a filter into a pump system or a syringe feed system as the filters can be added in-line. For this reason these systems both would be slightly better choices.

The pump system and the use of an immiscible liquid both would be fairly easy to use. A pump system would require the user to operate an injection pump which would fill the anode (bacterial chamber) and simultaneously empty used media through a check valve system. Using an immiscible liquid would require the user to pour in fresh media into the anode and then drain used media from the anode to maintain the liquid level. This system would require the user to keep the liquid at the correct level manually and would leave the system open to user error which could result in the loss of the immiscible liquid layer and failure of the system. The syringe injection system would work much like the system with the immiscible liquid. This system, however, necessitates the use of sharp needles which create a hazard to the customer that the other designs do not.

Both a pump system and syringe system are relatively intuitive. Both use simple technology with which many people are familiar and require simple maintenance which

could be easily performed by the user. The immiscible liquid design is less intuitive because the barrier which keeps out excess oxygen is a layer of liquid. While this may be just as effective, it does not have the same type of intuitive design displayed by the other systems.

For BioVolt's MFC to be widely adapted it is very important that the MFC is very reliable. Because the feed system is a part of the design which does not affect power production it is even more important that this aspect of design is very reliable. Because of the possibility of spillage, the immiscible liquid design does not adequately meet this design criterion. Both the syringe design and the pump system design have the potential of being reliable, but the syringe system has the need for syringe needles. These can break or become dirty which could cause the feed system to fail.

All three design choices are very low cost, so all options performed very well in this category. The syringe system necessitates the purchase of replacement syringes and needles, so it has a slightly higher cost system. For these reasons, summarized in Table 7, an injection pump system was implemented in the final design prototype.

Table 7 - Cell Feed System Decision Matrix

Category	Category Weight	Immiscible Liquid	Liquid Subscore	Pump System	Pump Subscore	Syringe Injection	Syringe Subscore
Isolation From Oxygen	10%	9	0.9	8	0.8	9	0.9
Isolation From Bacteria	10%	8	0.8	10	1	10	1
Ease of Use	25%	6	1.5	9	2.25	7	1.75
Intuitive Design	15%	7	1.05	9	1.35	10	1.5
Reliability	25%	8	2	9	2.25	8	2
Cost	15%	10	1.5	10	1.5	9	1.35
Final Score	100%		7.75		**9.15**		8.5

5.3.5 Proton Exchange Membrane

As the most influential design aspect that affects the cell performance, the proton exchange membrane (PEM) was chosen very carefully. A PEM was chosen for the final prototype based on evaluation of each of the possible options against the following criteria:

- Performance: The most important factor is performance; the performance of the constructed prototype depends upon the performance of the PEM.
- Fouling Factor: Since the PEM is not replaceable, the fouling factor determines how long the cell will last before it is affected by an inefficient PEM.
- Cost: PEMs are generally quite expensive. The PEM expense could determine if the MFC is cost effective to manufacture or not.

Based upon the design criteria, four options for membranes were chosen and compared in a decision matrix, as shown in Table 8. Nafion and Ultrex are competing brands of PEMs used typically in the electroplating industry and fuel cell industry. Drawbacks to these PEMs are that they are relatively expensive and, more so with the Nafion, tend to foul if in the presence of chlorine. Cellophane was indicated in some of the literature as an appropriate membrane, but little research could be found about using cellophane membranes in MFCs, because many recent research documents use either a Nafion or an Ultrex membrane. Salt bridges are simple to construct, and are quite inexpensive. Their drawbacks, however, include a high internal electrical resistance as well as generally poor reliability. Salt bridges were included in the decision matrix nonetheless because of their low expense and wide availability. Due to performance issues, the Nafion and Ultrex membranes were chosen as the desired PEM despite their high cost. Cellophane scored low overall and was subsequently not considered for use as a PEM. Salt bridges scored better than expected, but their lack of performance and general difficulty with use deemed them unacceptable for use in the final prototype. However, because of their low cost, salt bridges were used in the experimental prototypes.

The Ultrex membrane was chosen as the best option to use as the PEM partly because it is less susceptible to fouling than Nafion, but mainly because BioVolt found a distributor of Ultrex membranes that was willing to send a sample size membrane for use in the final prototype for no charge.

The following decision matrix compares the four PEM options ranked against different categories of design criteria.

Table 8 - Proton Exchange Membrane Decision Matrix

Category	Category Weight	Nafion	Nafion Subscore	Ultrex	Ultrex Subscore	Cellophane	Cellophane Subscore	Salt Bridge	Salt Bridge Subscore
Performance	40%	10	4	10	4	6	2.4	3	1.2
Fouling Factor	20%	8	1.6	9	1.8	5	1	1	0.2
Cost	40%	1	0.4	1	0.4	3	1.2	10	4
Final Score	100%		6		**6.2**		4.6		5.4

5.4 Performance

The performance of the MFC was monitored by observing the voltage as a function of time. The results are displayed in Figure 6. As seen in this figure, a semi-steady output was attained after two weeks of operation.

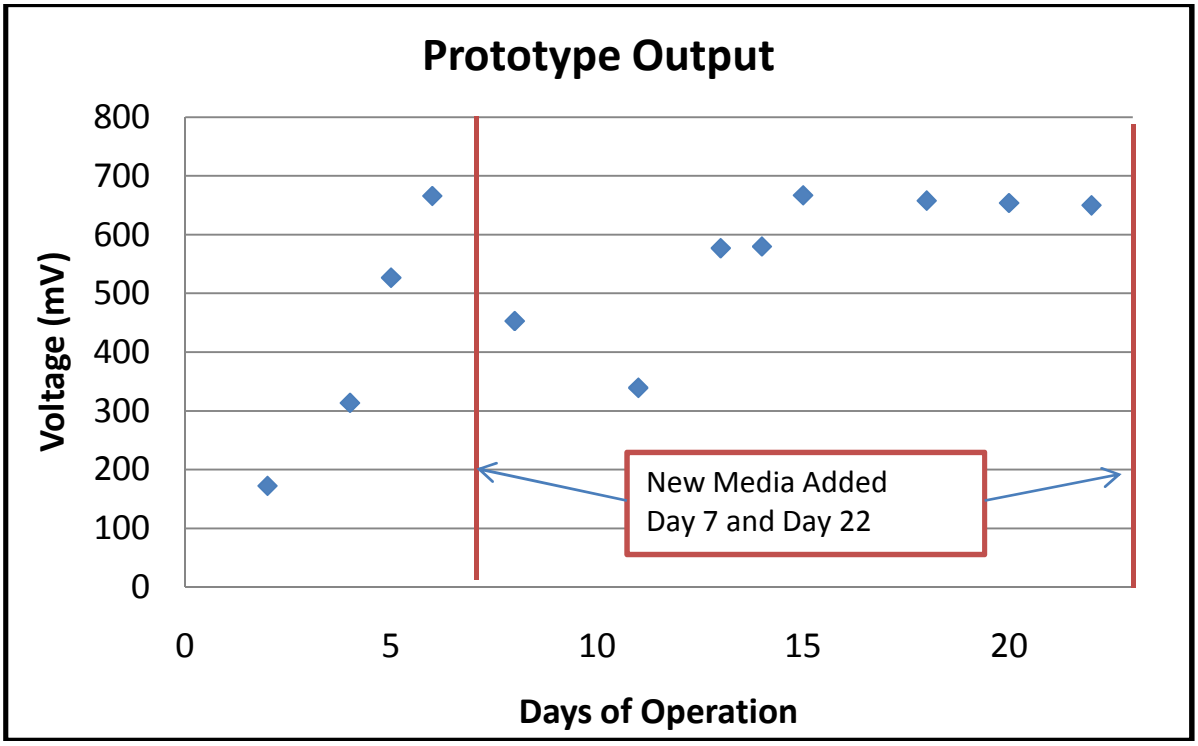


Figure 6 - Prototype Voltage Output

Media was added on day 7, as was suggested by the kinetics experiments to maximize bacterial growth on the electrodes. The addition of fresh media agitated and disturbed the forming biofilm, resulting in a temporary loss of electrical production for five days.

6 Conclusions

6.1 Current Design

The final MFC prototype was successful in producing a maximum voltage of 666 mV with a sustained voltage of 650 mV over a 979 kΩ resistance, yielding a power of about 0.5 μW. These preliminary results are projected to remain steady as the bacteria continue to thrive as the MFC is in operation.

A three chamber design was implemented: an anode chamber, cathode chamber, and media storage chamber. Inexpensive materials, such as graphite for electrodes and PVC for the casing material, were used to decrease cost to the project. The feed media was experimentally simplified into a mixture of baking soda, vinegar, table salt, ammonium chloride, and sodium phosphate.

6.2 Achieving Objectives

6.2.1 Sustainability

The objective was to generate sustainable electrical energy. BioVolt's MFC accomplishes this objective by generating power without the environmentally harmful and hazardous materials conventional batteries are composed of. The organic wastes produced by the MFC are the waste products of the bacteria breaking down the feed solution, and consists of carbon dioxide, unconsumed media, and water. The waste contains many nutritional components needed by plants and could be used for watering a garden. BioVolt's MFC contains microorganisms that reproduce and can produce power as long as there is a supply of fresh nutrients to the cell. BioVolt's MFC is designed to be reused, meaning upon bacterial death, the microorganisms and nutrient media can be replaced, providing new life to the cell. Furthermore, the materials of construction of BioVolt's MFC are completely recyclable if the user ever decides to dispose of the cell in its entirety.

6.2.2 Size

The objective was to produce a semi-portable design to comply with the culture of the area the MFC is intended to serve. The final prototype cell is indeed portable, with an overall size of two feet with a four inch diameter and weighing approximately fifteen pounds when all three chambers are completely full. However, the cell is designed to operate in a stationary position, since the cathode chamber must remain open to the atmosphere during operation.

6.2.3 Feed

The objective was to produce a feedstock that provided the required nutritional needs of the bacteria, but is inexpensive and also composed of readily available ingredients. BioVolt met this objective by optimizing the feed required by the bacteria, investigating replacement ingredients, and determining to what degree of feed ingredient measurement errors resulted in adverse effects on the MFC. The simplified formula is comprised of water, baking soda, vinegar, table salt, ammonium chloride, and sodium phosphate, all converted into easily measurable units such as teaspoons in order to simplify the construction of the feed media. All of these ingredients are considered readily available except for ammonium chloride and sodium phosphate. Since no readily accessible analogs for these salts could be found, it was determined that BioVolt would market their MFC as a kit that includes a year's supply of these components along with the MFC casing and microbial culture.

6.2.4 Lifetime

The objective was to produce a prototype that maximizes the life of an MFC with minimal user intervention. BioVolt accomplished this goal by designing an MFC that functions as a semi-batch process. The biological aspect of an MFC can theoretically last indefinitely, given the cell conditions are maintained and the microbes are supplied with sufficient nutrients. A semi-batch process is simpler than a constant flow nutrient feed, and lasts longer than a batch process because the media and its nutrients can be replenished. This semi-batch design requires a user to operate the pump bulb five times per day. This provides enough nutrient turnover to sustain life.

6.2.5 Power Output

The objective was to produce a power output of 10 mW/m^2 or greater, which would improve upon existing literature data. BioVolt produced about 21 $\mu W/m^2$, which is significantly less than the literature data. While the output is not comparable to the literature data, it was achieved without using a platinum catalyst and demonstrates a rugged, non-laboratory design that could be implemented commercially.

7 Recommendations for Future Improvements

The major drawback of BioVolt's prototype MFC is the low power production compared to the prototype's size. Different possibilities exist which could improve this design and increase the power output of the cell while maintaining or even decreasing overall size.

The most influential improvement which could be made is adding a catalyst to the cathode electrodes which could catalyze the reduction of molecular oxygen to water. Different catalysts could be researched; however, literature has shown platinum to be a highly effective catalyst for this reaction. Power production of 1,000 to 10,000 times that of BioVolt's prototype have been shown using graphite electrodes with platinum loadings as low as $0.5mg/cm^2$ (Trinh et al. 752). The surface area of cathode electrode used in BioVolt's prototype would require approximately 50mg of platinum, but the overall surface area of the cathode electrodes must be optimized with the platinum-loaded electrodes and may be significantly less or more than the surface area used in BioVolt's prototype. This research was not implemented in this project because the production of platinum-loaded electrodes was not feasible due to a lack of equipment at Calvin College for such production and the cost restraints of procuring platinum-loaded electrodes.

In addition to introducing a catalyst to the cathodic chamber, MFCs have been constructed which rely on a different reduction reaction occurring at the cathode (Du et. al. 13). By employing a different reduction reaction other than the reduction of oxygen to water, a higher voltage and power could be achieved. The highest reported power outputs have been realized using ferricyanide as the electron acceptor in the cathodic chamber. These reported power outputs reach as much as 100,000 times the output of BioVolt's

prototype. However, the use of an electron acceptor, such as ferricyanide, may incur negative consequences, such as health concerns or greater expense.

Biologically, the power production capabilities could be tuned by incorporating other species of bacteria and/or adapting the bacteria to function more efficiently. Research has shown that a cocktail of different bacterial species has the potential to increase output 10 to 100 times that of *Geobacter sulfurreducens* (Rabaey et. al. 294). While using a bacterial cocktail would increase the cost of a prototype due to multiple bacterial species must be purchased, it may not significantly increase the cost of a production model MFC. Further research in this area is needed, but is beyond the scope of BioVolt's project because the acquisition of several bacterial species was not possible while maintaining BioVolt's project budget.

Different graphite materials used in the construction of the anode electrodes, such as graphite foam, also show promise for increasing power production (Dumas 2495). This provides a much higher surface area per volume of the electrode and could increase power production by providing more surface area on which the bacteria is able to grow. Increasing the surface area of the electrode would increase the amount of bacterial biofilm and decrease the residence time of the media. Research is needed into how often the user would be willing to replace the media. By replacing the media more often, more power is achievable for a given volume.

For optimization to be achieved, further research could have been conducted into the necessary size ratio of each of the chambers, specifically the cathode and media storage chamber size compared to the anode chamber. It was assumed that each of the three chambers should be approximately the same size; however, if it is possible to decrease the relative size of one of these chambers without effecting power production, the overall power production per size of the final MFC (anode, cathode, and media storage chamber) could be increased.

Another experiment that would have been useful, had the resources been available, would have been a study of the power production as a function of MFC volume. It was assumed that the size of the MFC directly and proportionally affects the power output. But it is also possible that there is a maximum point, where the tradeoff between size and

efficiency is no longer directly related. This would have helped with the size selection of the final prototype.

Alternatively, other designs of microbial fuel cells could be investigated. BioVolt worked with the traditional two-chamber proton exchange MFC, but other designs such as a single chambered air cathode MFC show great promise. Commercially, MFCs are being researched and prototypes are being constructed that remove the living organism from the design completely. Sony Corp. unveiled a prototype that incorporates only the enzymes necessary to digest the feed (Physorg.com), eliminating the inherent drawbacks of working with a live bacterial culture.

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Appendix A – Research Prototypes

Since one goal for the final design was to optimize the feed so that the cell can operate utilizing cheap and readily available materials, there was a need for experimental prototypes that could be used to easily compare the effects changing different variables would have on the system. To provide this need of experimental prototypes, five identical cells were constructed as defined below.

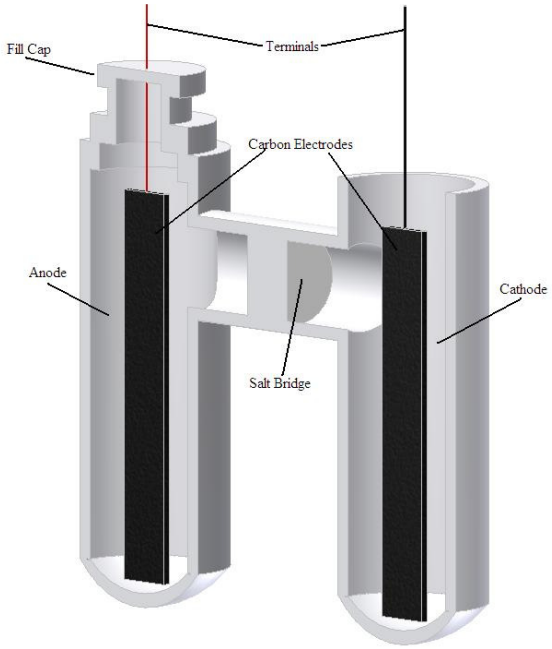


Figure A1 - Experimental Prototype Design

The experimental prototypes are built upon a classic "H" design that many researchers use for microbial fuel cells. The cells were constructed using 1 inch PVC piping and various PVC fittings. The pipes and fittings were glued together to prevent leaks and to prevent contamination from the environment. The pipe containing the salt bridge, however, was not glued to facilitate with the ease of changing the salt bridge between experiments.

Design Component Descriptions

Cathode Chamber: The Cathode Chamber holds the Cathode Electrode in an aqueous solution. The final reaction (reduction of oxygen) occurs in this chamber.

Cathode Electrode: The Cathode Electrode is composed of graphite since the material of construction was already decided before testing began in the experimental prototypes.

Salt Bridge: Since Proton Exchange Membranes are very expensive and fragile, an agar salt bridge was used instead. The salt bridge performs the same functions as a PEM would, but also is very inexpensive and easy to produce. The downside to a salt bridge is that it must always remain wet, fouls relatively quickly, and is not nearly as efficient as a real PEM is. Since the experimental prototypes need only to be compared to each other, using an inefficient salt bridge does not affect the ability to compare different variables being tested. The salt bridge (see appendix X for the recipe) is used only once before being replaced.

Anode Chamber: The Anode Chamber contains the Anode Electrode immersed in inoculated media. This chamber is nitrogen flushed and completely sealed off from the outside environment by means of a threaded plug.

Anode Electrode: The Anode Electrode is composed of graphite and is the location where the bio-film is formed by the microorganisms.

Appendix B – Bacteria and Media Testing and Optimization

Media Ingredient Substitution Experiment

Experimental Set-Up

Initial experiments were done to test the possibility of substituting supermarket available ingredients in the media. Having available ingredients is important to the cultural appropriateness of the project; if the materials for the feed are not easily attainable, upkeep of the MFC would be expensive and difficult.

The control media is the standard ATCC recipe attached in Appendix D, with five main ingredients, not including the vitamins and minerals. Of these five core components, two can be easily obtained with relative purity from a general supermarket. Specifically, sodium bicarbonate is purchasable in the form of normal baking soda, and sodium acetate is more commonly known as vinegar. A third substitution to be tested is the possibility of using common table salt, sodium chloride, instead of the recipe's potassium chloride. The necessity of potassium in the growth of the bacteria is not known and must be experimentally determined. Appropriate substitutions for lab grade ammonium chloride and dibasic sodium phosphate were not found, but each of these components is inexpensive. Also, another ingredient to test was the necessity of addition of sodium fumarate to the media after autoclave sterilization. It's noted that the addition of sodium fumarate is to act as an electron acceptor in the media (Kim et al., 71). Whether or not this is essential for short term growth was to be determined experimentally.

The substitution ingredients were purchased from Meijer and Aldi supermarkets. Morton salt was used for the sodium chloride, white distilled vinegar at 5% acidity was used for sodium acetate, and Arm & Hammer pure baking soda was used for the sodium bicarbonate.

Using the specified amounts in the ATCC recipe, four solutions were made up. The first was the control media, which followed ATCC specifications. The second media followed all ATCC specifications, but substituted with Morton salt instead of potassium chloride. The third media was substituted with baking soda instead of sodium bicarbonate, and the fourth used vinegar as a substitute for sodium acetate. To measure the vinegar, the solution was made up, and then brought down to a pH of about 7.0 using the vinegar. The pH of each solution was observed and verified to be 7.0 +/- 0.3. One quarter liter of each media was constructed using nanopure water followed by filter sterilization (process described in Appendix X)

Using 10 clean Balsch tubes, each substituted media was put into two tubes, but with four of the tubes filled with the control media. After being sterilized appropriately, vitamins and minerals were added to each of the tubes, and sodium fumarate was added to all but two of the tubes filled with control media. After inoculating with bacteria, the tubes were placed in the 30 °C incubator and grown for 10 days.

Results

After the 10 day growth period, the tubes were inspected qualitatively for growth. The results are as follows in Table .

Table A1 - Substitution Experiment Results

Tube	Growth appearance
1: Control	Very good growth- visible film
2: Control	Excellent growth- suspended filmy substance
3: Control media minus sodium fumarate	Ok growth- some floating clumps visible
4: Control media minus sodium fumarate	Very good growth- film covering bottom
5: Media substituted with salt	Ok growth- some floating clumps visible
6: Media substituted with salt	Excellent growth- suspended filmy substance
7: Media substituted with baking soda	Very good growth- film covering bottom
8: Media substituted with baking soda	Excellent growth- suspended filmy substance
9: Media substituted with vinegar	Ok growth- some floating clumps visible
10: Media substituted with vinegar	Excellent growth- suspended filmy substance

Conclusion

As seen in Table , all of the variations sustained adequate growth; enough to be visible in a bio-film form. This suggests that potassium is not essential in the growth of the Geobacter, nor is sodium fumarate. It is also encouraging to know substitutions with non-lab grade materials can be done with promising results equivalent to those done with the standard ATCC media. The results of this experiment also led directly to the next step of experimentation: total substitution.

Bacteria Kinetics Experiment

Experimental Set-Up

A key factor in the efficiency of the MFC is how long the bacteria will grow and how fast they will consume the food given. Also, temperature has a strong influence on the growth of bacteria, and the specific effects are important to understand to predict the robustness of the cell. Knowing these allows prediction of total MFC life and prediction of how often the MFC will need refilling for maintaining maximum growth and power.

Typically, a batch reactor containing bacteria follows a well established bacterial growth curve, as shown in Figure A2. There is a lag phase period, where growth and reproduction of the cells are just beginning, so initial growth is small. However, after

accustomed to the environment, if there are sufficient or excess nutrients, the bacteria will go through logarithmic growth. Reproduction will occur at an exponential rate. At some point in a batch, the nutrients and space available will be exhausted, and a stationary phase will be held by the bacteria. Here, while there is still reproduction, it only occurs approximately equal with the death rate. Finally, when all the nutrients have been consumed, the bacteria will enter the death phase, unless there is a change in the environment. The goal for optimum MFC performance is to have the user introduce fresh media containing nutrients in the latter part of the log phase.

This kinetic experiment was prepared in order to estimate these time parameters. Six tubes of standard ATCC media were prepared together, using the same techniques and procedures. Only 5 mL of media were put in each tube. Of these six tubes, four of them were inoculated at the same time. Two of the inoculated tubes were kept in the cupboard with one blank tube, and two were kept in a 30 °C incubator, also with one blank tube. Using the UV/Vis Spec20 spectrophotometer, the absorbance of the contents of the tube was monitored over the next 7 days at a wavelength of 600 nm. This is a typical wavelength used to measure the growth of various bacteria, and monitored at a similar wavelength, 620 nm, with well visible results (Dumas et al. 2495). This data gave a concentration profile mapping the growth of the bacteria. It also gave insight to the variance of growth due to temperature.

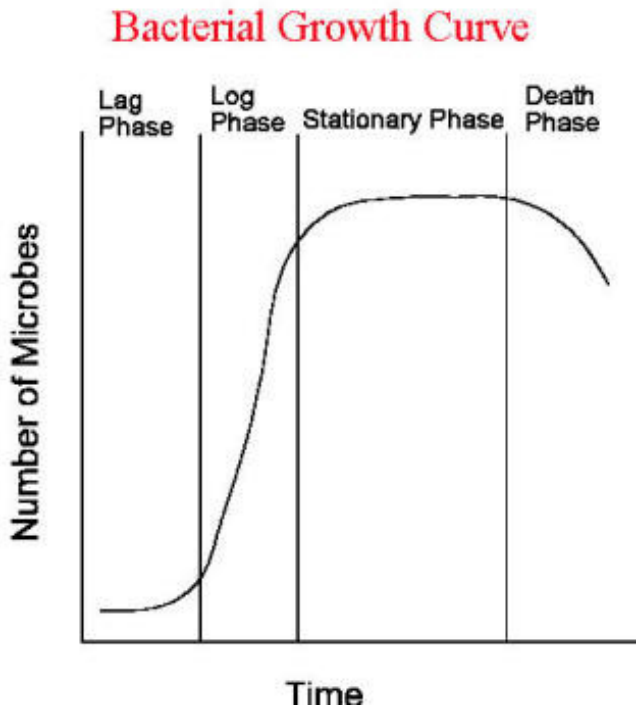


Figure A2 - Bacterial Growth Curve

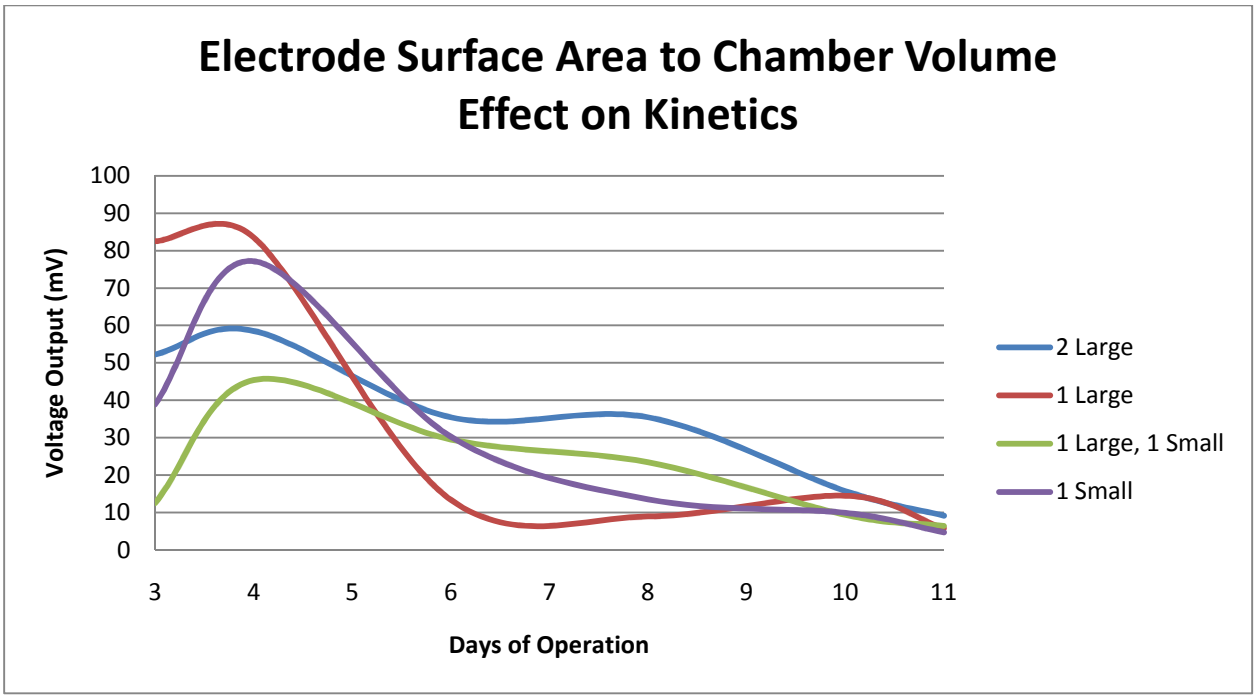


Figure A3 - SA/V Kinetics Data

Results

This absorbance data as a function of time was collected then was plotted using the integral method to determine the order of the reaction. A plot of the data from two of the tubes is shown in Figure A4, and a logarithmic plot of one of the runs is shown in Figure A5, depicting the integral method and displaying a first order reaction. It was also determined that the surface area to volume ratio that obtained the best results was the test with one large electrode in the research prototype chamber (approximately 1 in^2 : 1 in^3)

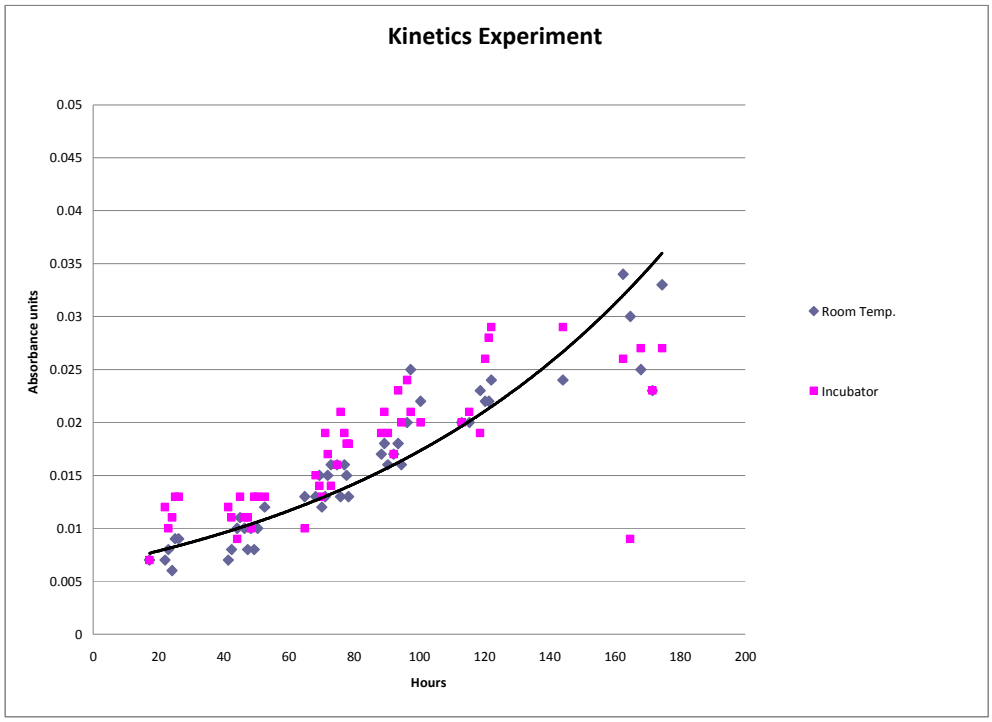


Figure A4 – Example of Kinetics Experiment Results

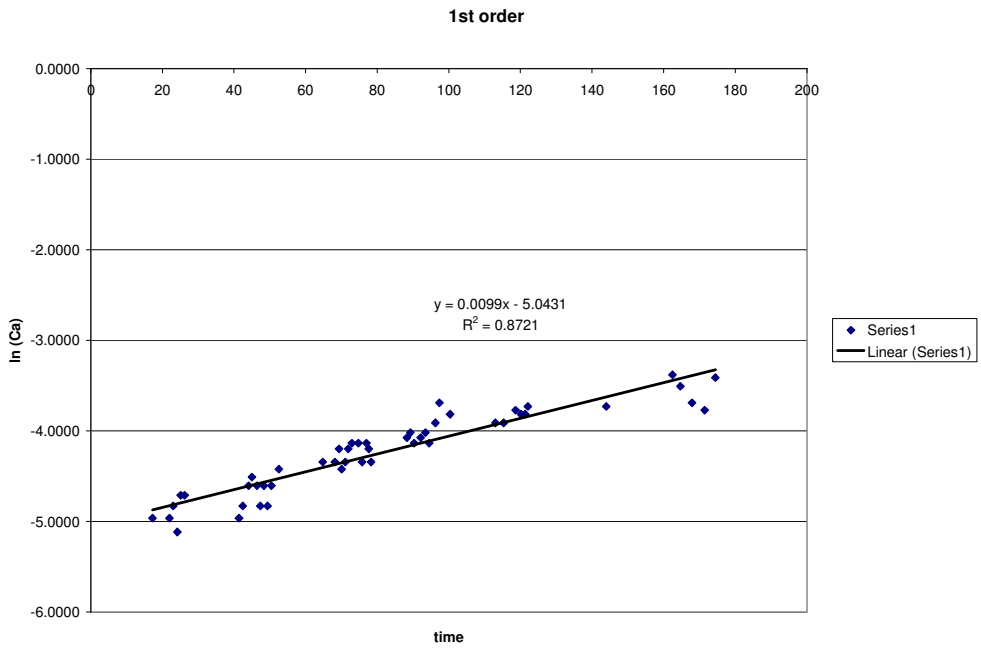


Figure A5 - Logarithmic Plot of Bacterial Growth

Conclusion

Each tube was found to be, as predicted, first order with respect to the bacteria, with specific growth rates determined using method of least squares. A first order reaction is in the following form:

$$Rate = \frac{dC_a}{dt} = k(C_a) \propto (k)(Abs)$$

where C_a (mol/L) is the concentration of the bacteria, which is directly related to the absorbance measured, and k (hours^{-1}) is the specific growth rate of the bacteria.

Combination of **Eq. 1** and a mole balance yields **Eq. 2** as follows

$$\ln(C_a) = kt$$

Using graphs such as Figure A5**,** the above equation can be plotted so k for each growth case can be solved. The calculated data is listed in Table A2 - Calculation of k (Temperature Test).

Table A2 - Calculation of k (Temperature Test)

	k (hours^{-1})	Standard Deviation
Tube 1. Room temp.	9.85 E-3	+/- 0.55 E-3
Tube 2. Room temp.	11.77 E-3	+/- 0.75 E-3
Tube 3. Incubator	16.47 E-3	+/- 1.56 E-3
Tube 4. Incubator	6.30 E-3	+/- 0.83 E-3

This data is somewhat surprising in that it is difficult to tell the temperature effect on the growth rate. It was expected and assumed that an increase in temperature would yield a faster growth rate, as tube 3 in Table show when compared to tubes 1 and 2. However, tube 4, whether due to extraneous variables, error, or natural causes, was the slowest growing of the test. With only these two tests at different temperatures, it's hard to infer a relationship with temperature. However, it is reasonable to assume based on this data that the MFC would function sufficiently in most ambient temperatures.

However, the specific growth rates are accurate enough to give a general picture of growth of the Geobacter under standard conditions. The average k = 11.10 E-3 (hours^{-1}) when the concentration is found using absorbance units. From other old samples, it was

noted that the highest absorbance reached when the tube was cloudy with bacteria was about 0.10. Using an ordinary differential equations solver program to graph **Eq. 2**, Figure A6 was created as a prediction of the logarithmic growth stage of the bacteria. It is predicted that the bacteria will reach the end of the log growth stage around 240 hours, or approximately 10 days, in 5 mL of media in a static environment. Therefore, if would be appropriate to introduce fresh, nutrient rich media to the MFC about one every two weeks. This would allow for logarithmic growth to occur and the food of the cell to be used up. However, instead of allowing the cells to reach a stationary phase, the addition of nutrients in the form of fresh feed would allow for the bacteria to reenter a prosperous stage.

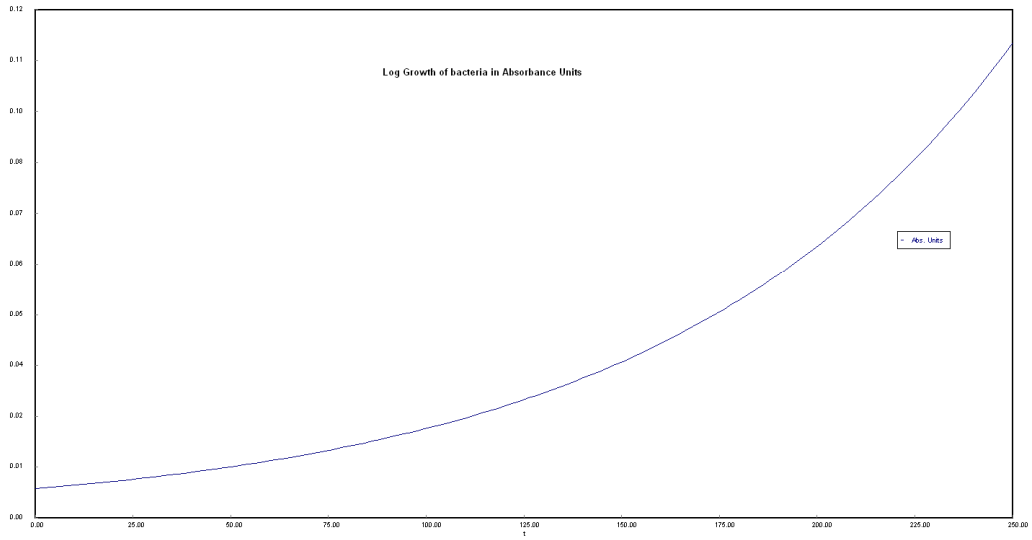


Figure A6 - Prediction of Logarithmic Growth Rate

In order to extrapolate beyond an environment containing 5 mL media of nutrients, the absorbance must be converted to a concentration of cells. This is done using Beer's Law,

$$Abs = C_{\alpha} \varepsilon L$$

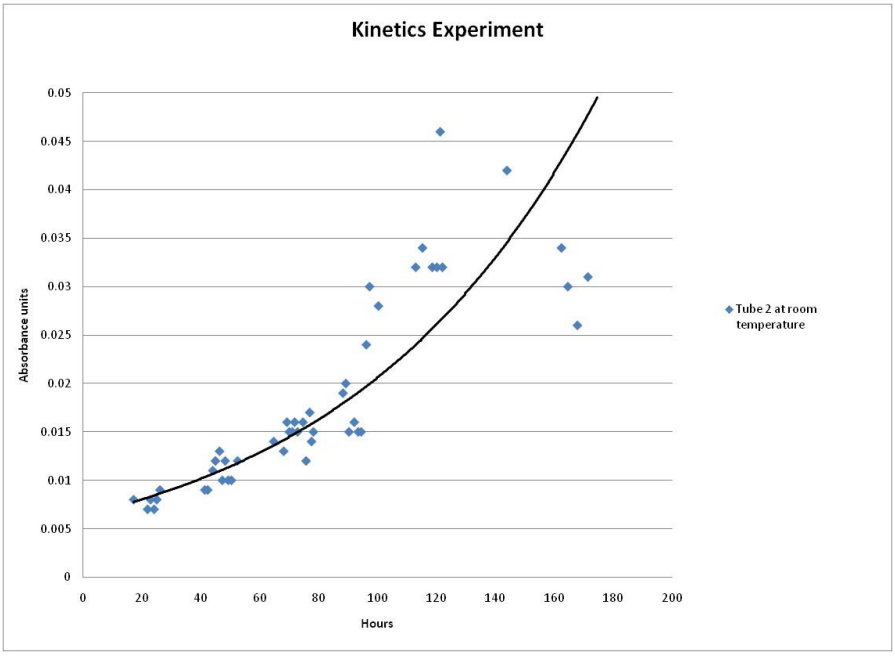
where *Abs*, the absorbance, is equal to the concentration, in molarity, multiplied by the extinction coefficient (ε) of the bacteria, multiplied by the length (*L*) of the sample the beam of the Spec20 passes through. In this case, and extinction coefficient was estimated to be approximately 30,000 $M^{-1}\,cm^{-1}$. This was projected as Geobacter is similar to E. coli, and an average E. coli extinction coefficient is in the upper 20,000 through 40,000 $M^{-1}\,cm^{-1}$. The diameter of the Balsh tube was 1.5 cm.

A main concern that propagated error is in the measuring of the bacteria once it began to form a bio-film. When using the UV/Vis, the measurement is taken at a specific

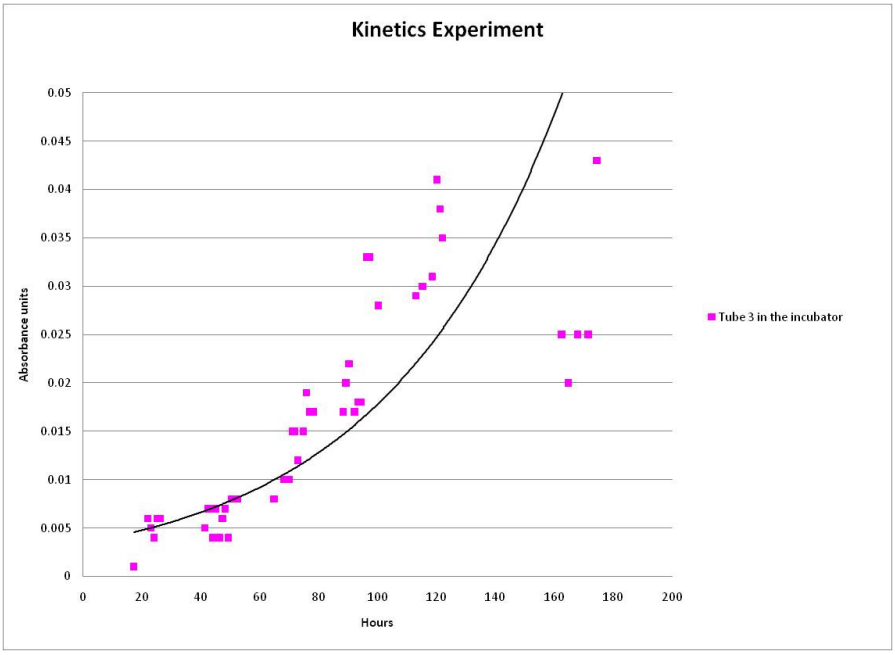
point in the tube. If a clump of bacteria in a bio-film were to cross the path of the light, the absorbance of the clump would be taken. However, if no clump were to pass and only free floating bacteria were to pass through the measuring area, a much smaller absorbance would be displayed. Therefore, the absorbance measurements later on in the experiment begin to increasing vary, as some measurements are taken of clumps and some of suspended single microbes.

Kinetics Data

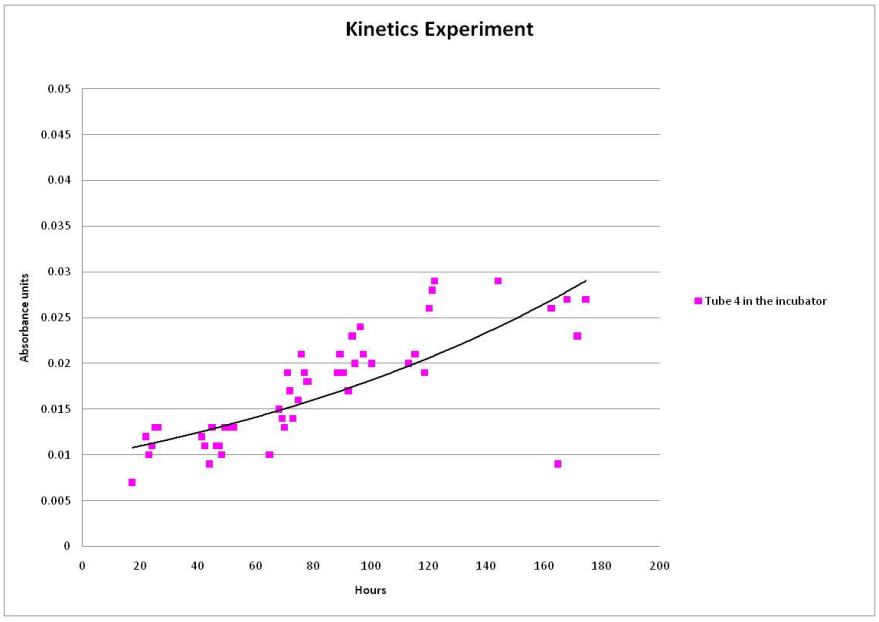
Kinetics Experiment Results for tube 2 at room temperature.



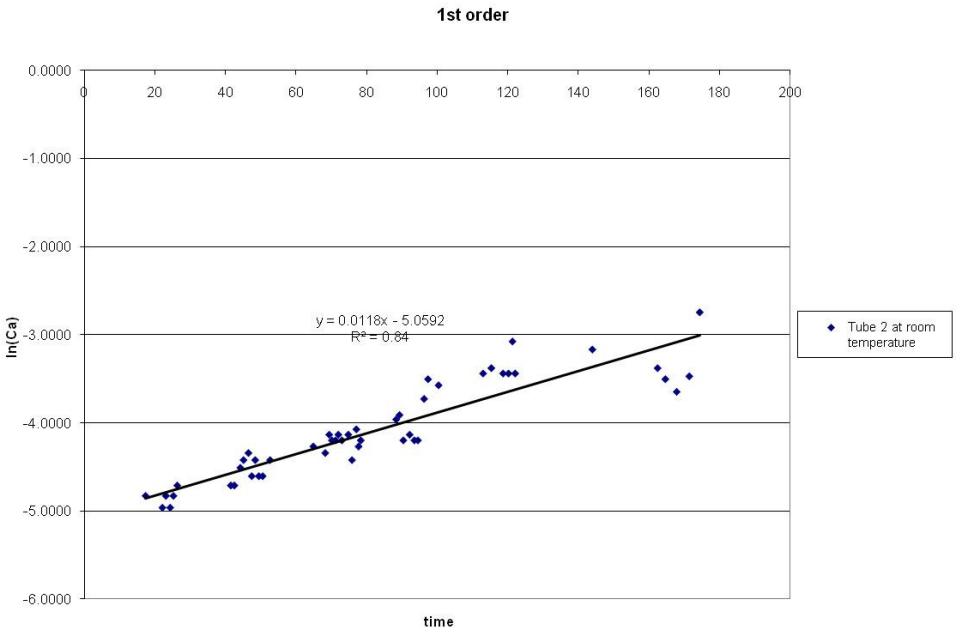
Kinetics Experiment Results for tube 3 in the incubator.



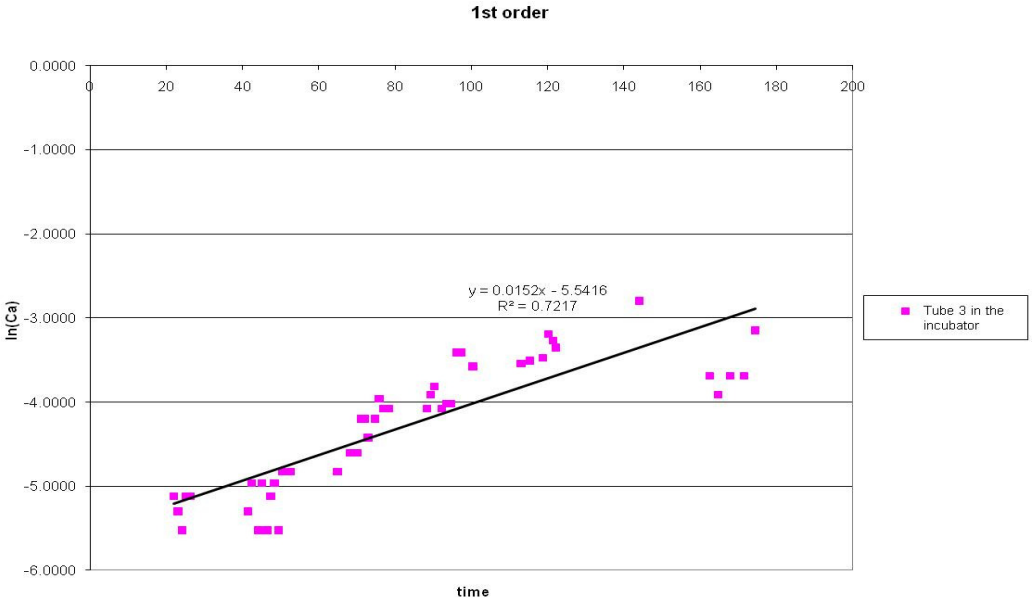
Kinetics Experiment Results for tube 4 in the incubator.



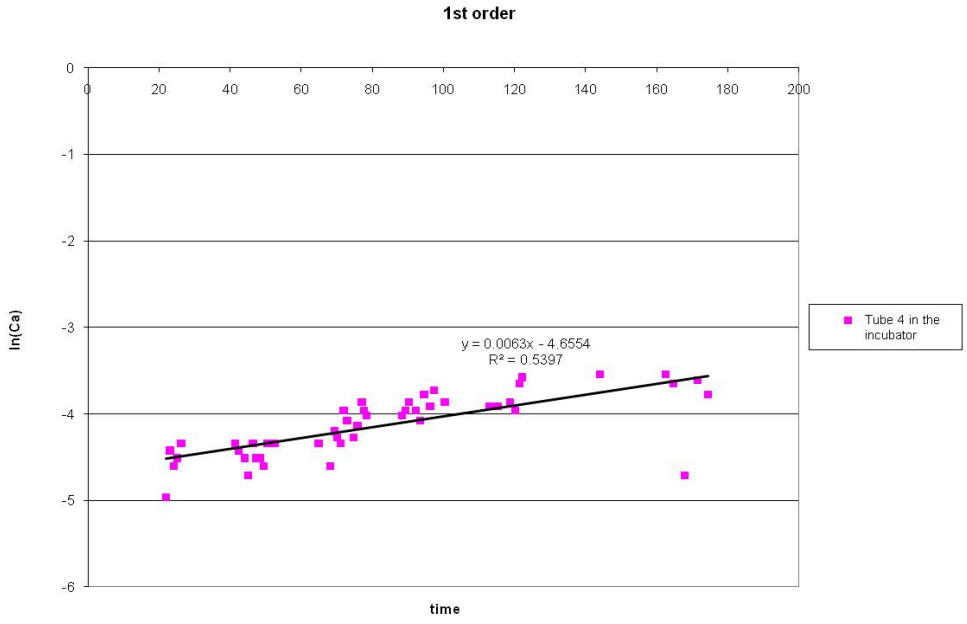
Logarithmic plot of bacterial growth tube 2 at room temperature.



Logarithmic plot of bacterial growth tube 3 in the incubator.



Logarithmic plot of bacterial growth tube 4 in the incubator.



Media Ingredient Omission Experiment

Experimental Set-Up

Although three of the original lab grade ingredients had been substituted and one ingredient was deemed unnecessary, there were still two components, dibasic sodium phosphate and ammonium chloride, which did not have close substitutes outside the laboratory. The following experiment was created to test how crucial these components were to the health and growth of the bacteria.

Four different media were mixed, each made with the tested substitutable ingredients (i.e. vinegar, baking soda, and salt). However, three of the four media were each lacking one or both of the phosphate or chloride components. The first media was a control, which contained the ATCC specified amount of phosphate and ammonium chloride. The second media did not contain any phosphate component, and the third did not contain any ammonium chloride. Finally, the fourth media did not have phosphate or ammonium chloride, but only the substitutable ingredients. Each media was dispersed into two tubes of 5 mL each and all were inoculated at the same time. All the tubes were placed in the 30 °C incubator for two weeks.

Results

Of all the tubes set to grow, the only ones that displayed any growth were tubes containing the control media. None of the omission tests were successful.

Conclusion

It can be concluded that phosphates and ammonium chloride are essential for the growth of the bacteria. This is not surprising, as all living organisms require phosphates for DNA and ATP production. Nitrogen is also a component that catalyses growth on a bacterial level. Because of this, it will be necessary to include phosphate and ammonium chloride in a package, as these components are not readily available in remote places. However, these are not expensive, nor are they restricted or hazardous materials.

Robustness

Experimental Set-Up

As the media will be made and mixed in non-laboratory setting with non-laboratory measuring devices, it is very likely that some measurement error will occur in the media. It is important to understand if these types of errors will affect the health and growth of the bacteria. For example, the addition of too much 5% acidity vinegar (about 1 mL or more) would affect the pH of the solution to the point that the bacteria could not survive in such acidic conditions. Ammonium chloride is also acidic, with a pH of 5.5. Optimum growth pH is about 6.8-7.0. So the addition of too much vinegar would result in cell death, possibly killing all the bacteria in the cell. But imperfect media in salt is not as detrimental. In order to prevent bacteria stress and death with other components, a robustness test was designed. Since the result of too much of vinegar and ammonium chloride is predictable, they were not tested.

The overuse of three materials was tested: table salt, baking soda, and phosphate. First, a control of the fully substituted media was made. Then, additional tubes containing 2, 5, 10, and 100 times the ATCC suggested amount of the ingredient were made up and inoculated. The tubes were placed in the 30 °C incubator for two weeks.

Results

Table summarizes the results of the experiment.

Table A3 – Robustness Test, Experimental Results

	NaCl	Baking Soda	Phosphate
Control	Growth	Growth	Growth
X 2	Growth	Growth	None
X 5	Growth	Growth	None
X 10	Growth	None	None
X 100	None	None	None

Conclusion

As seen by Table 9, a large excess of salt still yields visible growth. In fact, it is possible that salt stressed bacteria are more likely to form a stronger bio-film. Quite a bit of excess baking soda is allowable for still healthy bacteria; however, any additional phosphate seems to hinder the growth of the bacteria. It is possible that the bacteria were still growing in the excess phosphate solution but were not at the visible amount. Typically, the number of bacteria must be on the order of 10^6 before they are visible to the naked eye. Because of the death or stunted growth of the bacteria under non-ideal conditions, the user's manual or instructional reading material accompanying the MFC must note the effect of poorly or incorrectly mixed media. This experiment also confirmed that optimal performance seems to be at the ATCC suggested amounts or ingredients in the media.

Appendix C – Basic procedures for handling bacteria

Tubing the Anaerobic Media

The following procedure was followed to ensure optimal bacterial growth. Leaving the media on vacuum removes dissolved oxygen from the solution. Flushing the media and tubes with nitrogen reduces the exposure to air, as well as attempts to create an air free, anaerobic environment within the test tube.

1. Pour excess of the desired media amount into a vacuum Erlenmeyer flask. Place stopper in position and place the media in a sterile environment with a vacuum system. Vacuum the media for 20-30 minutes.

2. Quickly transfer media from vacuum flask to an Erlenmeyer flask under a flow of nitrogen. Insert stopper into flask, and flush with nitrogen for two minutes.

3. While flushing the media, flush a clean Balsh tube with nitrogen for two minutes as well.

4. Using a sterile pipet, transfer 5 mL of media into Balsh tube. Be sure to minimize the time any of the media is exposed to air.

5. Once the media is in the Balsh tube, flush the tube again with nitrogen for two minutes.

6. Insert rubber stopper fully, cap with metal pull-back top, and crimp with a metal fitting.

7. Autoclave using the Liquid20 program.

Prepping for bacteria

This procedure was followed in the early stages of testing to ensure initial bacterial growth and culturing. The desired final design, however, excludes the addition of sodium fumarate, vitamins, and minerals for cost and simplicity purposes, as these ingredients are not crucial for the survival of the culture.

1. Sterilize the injection sites by swabbing the tops of the Balsh tubes with 75% ethanol followed by flame.

2. Using a 1 mL sterile syringe with sterile tip, add 0.5 mL of sodium fumarate to the tubes to be inoculated. Take care to remove air bubbles from syringe before injecting into the tube. Also, tubes should be tipped upside down to maintain a seal as the syringe is inserted and removed.

3. Repeat this procedure when adding 0.1 mL of Wolffe's vitamin solution and 0.1 mL of Wolffe's mineral solution.

Bacteria transfers

The following procedure was used when transferring bacteria into experimental tubes or further culturing tubes. The grown bacteria tube should contain bacteria grown for at least five days, but no longer than two weeks. The tube to be inoculated should have been Autoclave sterilized to ensure no competing bacteria.

1. Sterilize the injection sites on both the tube with grown bacteria and the tube to be inoculated. Swab the tops with 75% ethanol followed by flame.

2. Shake the tube containing the bacteria, as a biofilm of cultures will have formed on the bottom of the Balsh tube. Tip the tube upside down when inserting sterile, 1mL syringe with sterile tip.

3. Draw up about 1 mL of bacteria/media. Remove syringe with tube still tipped to maintain a seal.

3. Insert syringe into an inverted, prepped tube and inoculate with all the contents of the syringe. Take care to remove air bubbles from the syringe.

4. Place in the 30 degree Celsius incubator for best results.

Filter Sterilizing

This procedure is to be done in a sterile environment. It was followed after mixing of a new media as an alternative to Autoclave sterilization. The VacuCap contains a filter of 0.22 µm, which filters out dirt and undesired bacteria.

1. Spray gloved hands and the hood surface with IPA. Allow it to evaporate. Also spray the bottoms of all beakers and bottles placed in the hood.

2. Remove VacuCap from packaging. Do not touch the bottom surface of the cap, as it must remain sterile.

3. Attach vacuum tubing to vacuum valve on the cap. Attach free tubing to valve labeled 'inlet' on the cap.

4. Place the free end of the inlet tubing in media solution.

5. Place cap firmly on sterile glass storage bottle.

6. Turn on vacuum until all media has filtered through the VacuCap and is in the sterile glass bottle.

Freezing bacteria

This procedure was only done once as a method of long term storage for samples of bacteria. The frozen storage of back up bacteria was kept in case of emergency need of cultures.

1. Remove metal cap and rubber stopper from Balsh tube.

2. Quickly transfer media containing bacteria into a sterile centrifuge tube and flush with nitrogen. Remember to shake the Balsh tube before pouring to loosen the biofilm of bacteria formed on the bottom of the tube. Also, do this step quickly to minimize bacteria exposure to air.

3. Centrifuge tube on 3000 rpm for 20-30 minutes.

4. Bacteria should be visible as a small pellet on the bottom. Discard all but 1 mL of media from the centrifuge tube, attempting not to disturb the pellet.

5. Add 0.27 mL of 75% glycerol to milliliter of solution. Shake well.

6. Transfer bacteria media glycerol solution into sterile cryotube using sterile pipet.

7. Cap cryotube and store in -80°C freezer.

Bacteria disposal

This procedure was followed for the disposal of all bacteria.

1. Autoclave old bacteria on program Liquid20.

2. Remove metal capping.

3. Remove rubber stopper and place in biomaterial trash.

4. Dump sterile contents in drain. Clean tubes as usual.

Appendix D – ATCC Media Recipe

NH_4Cl	1.5 g
NaH_2PO_4	0.6 g
KCl	0.1 g
$NaHCO_3$	2.5 g
Sodium acetate	0.82 g
Sodium fumarate (filter-sterilized)	8.0 g
Wolfe's Vitamin Solution (see below)	10.0 ml
Modified Wolfe's Minerals (see below)	10.0 ml
Distilled water to	1.0 L

Prepare and dispense medium without fumarate anaerobically under an atmosphere of 80% N_2, 20% CO_2. Autoclave at 121C for 15 minutes. Add fumarate from a filter-sterilized, nitrogen-sparged stock solution prior to inoculation. Final pH of medium should be approximately 6.8.

Wolfe's Vitamin Solution:
Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no.MD-VS).

Biotin	2.0 mg
Folic acid	2.0 mg
Pyridoxine hydrochloride	10.0 mg
Thiamine .HCl	5.0 mg
Riboflavin	5.0 mg
Nicotinic acid	5.0 mg
Calcium D-(+)-pantothenate	5.0 mg
Vitamin B12	0.1 mg
p-Aminobenzoic acid	5.0 mg
Thioctic acid	5.0 mg
Distilled water	1.0 L

Modified Wolfe's Minerals:

Na_2SeO_3	10.0 mg
$NiCl_2 .6H_2O$	10.0 mg
$Na_2WO_4 .2H_2O$	10.0 mg
Wolfe's Mineral Solution (see below)	1.0 L

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no.MD-TMS.)

Nitrilotriacetic acid	1.5 g
$MgSO_4 .7H_2O$	3.0 g
$MnSO_4 .H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 .7H_2O$	0.1 g
$CoCl_2 .6H_2O$	0.1 g
$CaCl_2$	0.1 g
$ZnSO_4 .7H_2O$	0.1 g
$CuSO_4 .5H_2O$	0.01 g
$AlK(SO_4)_2 . 12H_2O$	0.01 g
H_3BO_3	0.01 g
$Na_2MoO_4 .2H_2O$	0.01 g
Distilled water	1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

Appendix E –MSDS – Ammonium Chloride

Material Safety Data Sheet
Ammonium chloride MSDS

Section 1: Chemical Product and Company Identification

Product Name: Ammonium chloride

Catalog Codes: SLA3415, SLA1069, SLA2575, SLA4078, SLA1732, SLS3118

CAS#: 12125-02-9

RTECS: BP4550000

TSCA: TSCA 8(b) inventory: Ammonium chloride

CI#: Not applicable.

Synonym: Ammonium Chloratum; Ammonium Chloridum; Ammonium Muriate; Sal Ammonia; Salmiac

Chemical Name: Ammonium Chloride

Chemical Formula: NH4Cl

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call: 1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Ammonium chloride	12125-02-9	100

Toxicological Data on Ingredients: Ammonium chloride: ORAL (LD50): Acute: 1650 mg/kg [Rat.]. 1300 mg/kg [Mouse].

Section 3: Hazards Identification

Potential Acute Health Effects:
Hazardous in case of eye contact (irritant). Slightly hazardous in case of skin contact (irritant, sensitizer), of ingestion, of inhalation.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.
Repeated or prolonged exposure is not known to aggravate medical condition.

Section 4: First Aid Measures

Appendix F – MSDS – Sodium Phosphate Monobasic MonoHydrate

Material Safety Data Sheet
Sodium phosphate monobasic monohydrate MSDS

Section 1: Chemical Product and Company Identification

Product Name: Sodium phosphate monobasic monohydrate

Catalog Codes: SLS1165, SLS4009

CAS#: 10049-21-5

RTECS: WA1900000

TSCA: TSCA 8(b) inventory: No products were found.

CI#: Not available.

Synonym: Dihydrogen Sodium Phosphate; Sodium dihydrogen phosphate monhydrate; Sodium Biphosphate monohydrate; sodium dihydrogen orthophosphate monobasic; Sodium Phosphate, Monobasic, Hydrate

Chemical Name: Sodium Phosphate, Monobasic, Monohydrate

Chemical Formula: NaH2PO4.H2O

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call: 1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Sodium phosphate monobasic monohydrate	10049-21-5	100

Toxicological Data on Ingredients: Sodium phosphate monobasic monohydrate LD50: Not available. LC50: Not available.

Section 3: Hazards Identification

Potential Acute Health Effects: Slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

Potential Chronic Health Effects: CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4: First Aid Measures

Eye Contact: Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at

MFC-V1 – User Manual BioVolt

USER MANUAL

MFC-V1

Congratulations on becoming an owner of the MFC-V1 by BioVolt. We at BioVolt have taken great care in making sure that this product performs to your expectations.

The MFC-V1 has been designed by BioVolt to operate, with only limited maintenance, for over 1 year. It provides an environmentally friendly sustainable power source which can be used to fulfill a variety of small scale electrical needs.

With the MFC-V1 you can charge the battery on a medical device, recharge your cell phone, or run LED lights to light up your house!

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2

1 INTRODUCTION

The MFC-V1 by BioVolt is a microbial fuel cell which has been designed to produce enough power to light low power LED lights and charge batteries for use in medical equipment, emergency cellular telephones, or other electronic devices.

A microbial fuel cell (MFC) is a "battery" which produces power using a specific species of bacteria. This bacteria eats a specific food solution, called the bacterial media, and produces electrical power which the MFC-V1 harnesses.

More information about your new MFC can be obtained by calling or emailing BioVolt at:

1-800-BIOVOLT

service@biovolt.org

or find us online at

biovolt.org

***NOTE: ALL CONTACT INFORMATION IS FOR DEMONSTRATION PURPOSES FOR THIS DOCUMENT ONLY AND IS IN NO WAY AFFILIATED WITH BIOVOLT OR ANY OF ITS TEAM MEMBERS.**

2 INCLUDED WITH THE MFC-V1

2.1 BACTERIA

The species of bacteria included with the MFC-V1 is called *Geobacter sulfurreducens*. Proper care instructions are outlined in **5 CARING FOR THE BACTERIA**.

2.2 FEED MATERIALS

- Sodium Phosphate Monobasic
- Sodium Phosphate Dibasic
- Ammonium Chloride
- Baking Soda (if requested)
- Non-Iodized Salt (if requested)
- White Vinegar (if requested)

2.3 PARTS AND TOOLS

- 12 replacement filters (Part #009)

MFC-V1 – User Manual BioVolt

- 1/8 inch hex key (Part #018)
- Measuring spoons (if requested)

2.4 WARRANTY

If at any time within the first year of ownership you experience any manufacturing default, you may send it back and BioVolt will fix or replace it at no charge to you. Also, if at any time the bacteria die of natural causes or your MFC-V1 ceases operation, BioVolt will replace it at no cost (user pays shipping and handling charges).

Modifications to this product, or operation of this product in any way other than outlined in this manual, could void the manufacturer's warranty.

3 ENVIRONMENT, HEALTH, AND SAFETY

3.1 ENVIRONMENT

The operation of the MFC-V1 is environmentally friendly as it is utilizing natural processes to create your power. The bacteria waste is in two forms: the used liquid media and CO_2 gas. A small amount of CO_2 is produced as the bacteria consume the nutrients, and this gas waste is expelled from the anode chamber as you refill or pump in new media. The used media that exits the chamber is only a salt solution, and can be disposed of with no harm to the environment.

3.2 HEALTH

Most of the ingredients in the media solution are kitchen appropriate and pose no harm if handled or ingested. If consumed in

large quantities, patient may experience some discomfort. The sodium phosphate and ammonium chloride are less common items, but also pose no health threats due to exposure to the user. Ammonium chloride and phosphate are noted as mild skin irritants and the chloride is slightly acidic when dissolved in water, so should be handled with care. Should you experience any irritation or discomfort, wash area with soap and water. Seek medical attention if further irritation develops. The MSDS sheets for these compounds are included.

3.3 SAFETY

Care should be taken when working with any device that carries an electrical charge. Proper care for connecting and disconnecting an electrical device to the MFC-V1 should be taken.

4 PRODUCT OVERVIEW

4.1 HOW IT WORKS

The MFC gets its electricity by harnessing the metabolism of a certain type of bacteria, *Geobacter sulfurreducens*. This bacteria "eats" the food solution, called the media in biological terms, and gives off electrons as shown in **Figure 1** below.

By continuing to feed the bacteria the prescribed media, bacteria in the cell can grow and continue to produce electricity. The bacteria are fed by filling the media storage chamber with fresh media and injecting the new media into the anode chamber via the injection pump system. Any waste media is then discarded out of the waste stream.

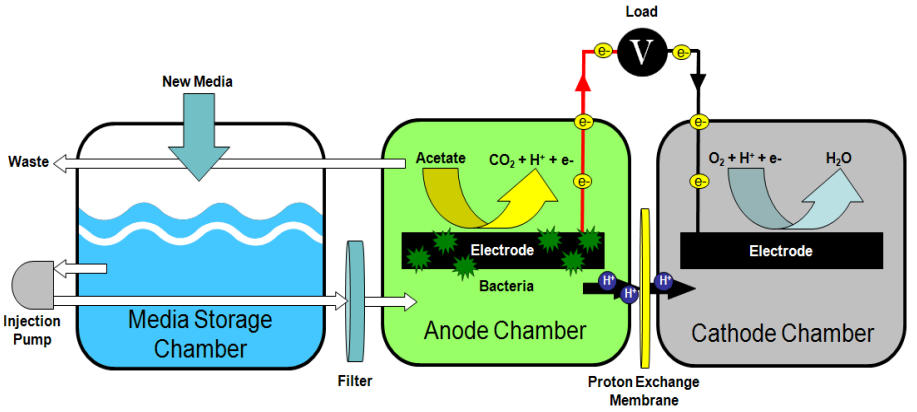

Figure 1. Schematic showing the biology and operation of the MFC-V1

4.2 CELL LAYOUT

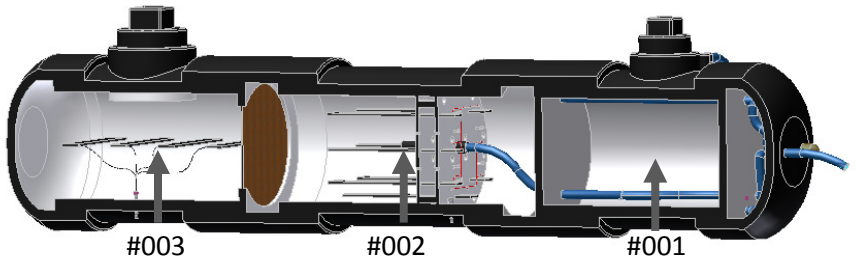

Figure 2. Full cell cutaway with part numbers

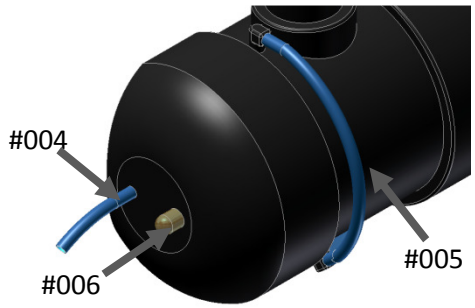

Figure 3. Cell end cap

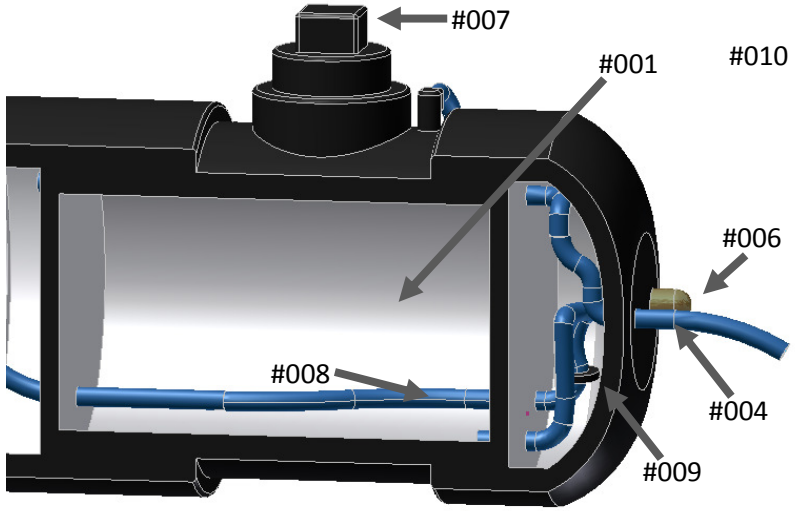

Figure 4. Injection apparatus close-up view

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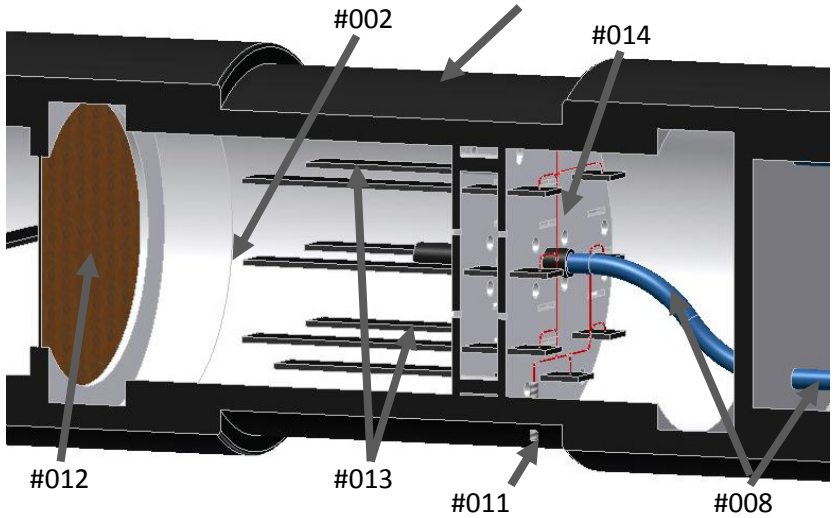

Figure 5. Anode chamber close-up view

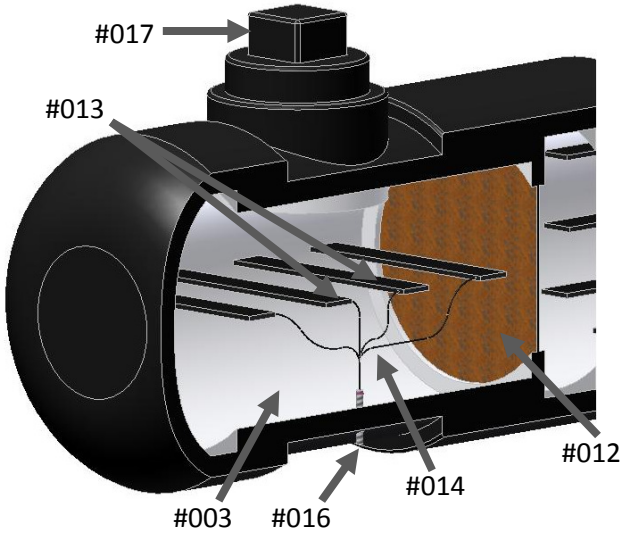

Figure 6. Cathode chamber close-up view

4.3 PARTS LIST

001 Media Storage Chamber
002 Anode Chamber
#003 Cathode Chamber
#004 Waste Outlet
#005 Level Indicator
#006 Injection Pump
#007 Media Storage Chamber Fill Cap
#008 Injection Hose
#009 Bacterial Filter
#010 Media Storage Chamber End Cap
#011 Anode Terminal
#012 Proton Exchange Membrane
#013 Graphite Electrodes
#014 Insulated Copper Wire
#015 Anode Fill Plug
#016 Cathode Terminal
#017 Cathode Chamber Fill Cap
#018 Drain Hex Key

5 CARING FOR THE BACTERIA

5.1 HEALTH CONSIDERATIONS

Several simple steps are necessary for *Geobacter sulfurreducens*, the bacteria used in the MFC-V1, to remain in peak health:

- Always follow the media recipe accurately
- Ensure only clean water is used in the MFC
- Never leave the anode chamber open to air
- Avoid exposure of the bacteria to direct sunlight
- Follow all procedures outline in this manual

5.2 NUTRIENTS

The media solution has been specifically designed for the optimal health of the bacteria as well as optimal power output. The feed for the bacteria is vinegar, the main component of which is called acetate. The bacteria digesting vinegar creates the power of the MFC-V1.

Sodium phosphate acts as a phosphate source and ammonium chloride acts as a nitrogen source to promote bacterial growth and health.

Baking soda makes the media less acidic so that the bacteria is able to survive in the media.

Table salt provides the bacteria with a source of sodium which helps to promote bacterial health.

6 GETTING STARTED

6.1 INITIAL SETUP

After receiving the cell follow the instructions for making media given in **7.1 MEDIA RECIPE**. Make a 3x batch of media to initially fill the anode and media storage chamber. Fill the anode chamber (Part #002) half full with media by removing the anode fill plug (Part #015).

Add provided bacteria to cell. Fill the rest of the chamber with media trying to leave as little air space as possible. Replace fill plug. Fill the media storage chamber with the remaining media. Inject media using the injection pump until some volume of waste is removed with each pump.

Once the anode chamber is full, follow the instructions outlined in **7.2 BUFFER RECIPE**. Make one batch of buffer and add it to the cathode cell (Part #003). Leave the cathode fill cap (Part #017) off unless transporting the cell.

The MFC is now ready to begin the bacterial growth phase.

6.2 STARTING BACTERIAL GROWTH

Initial bacterial growth takes 12 days. Promote bacterial growth by connecting the anode terminal (Part #011) to the cathode terminal (Part #016) using a wire. Allow the cell to stand for 7 days

without feeding. After 7 days, inject 30 pumps of new media. Allow cell to stand for another 5 days without feeding. After 5 days, remove the wire connecting the terminals.

The MFC is now fully operational. Attach the positive and negative terminals (Part #016 and Part #011) to the desired load and begin operation under the procedure outlined in **7 GENERAL OPERATION**.

7 GENERAL OPERATION

7.1 MEDIA RECIPE

For the media to serve as an acceptable food source for the bacteria it must be made following these instructions:

1. Heat 1 liter of water to a boil
2. Let water cool to room temperature
3. Add:
   1/4 teaspoon Ammonium Chloride
   1/8 teaspoon Sodium Phosphate Monobasic
   1/2 teaspoon Baking Soda
   1 teaspoon White Vinegar
   A pinch Non-Iodized Salt
4. Mix until all ingredients are dissolved

7.2 BUFFER RECIPE

The cathode buffer is prepared as the following recipe:

1. Measure 2 liters of water
2. Add 3/8 teaspoons of Dibasic Sodium Phosphate

7.3 Feeding the Bacteria

Verify that the media in the media storage chamber (part #001) is not cloudy. Cloudy media indicates that it has been contaminated by other bacteria. If media is cloudy discard immediately. Make sure the level indicator (Part #005) on the side of the cell shows sufficient media in the media storage chamber. Pump the media from the media storage chamber into the anode chamber (Part #002) where the bacteria is growing using the injection pump (Part #006).

The optimal injection rate is 5 pumps per day to maintain adequate food levels. The bacteria can survive up to one week without food before showing any adverse effects. If left unattended for a number of days, inject all food which had been missed, in addition to food for the current day (i.e. 10 pumps if 1 day missed, 25 pumps if 4 days missed).

Verify that the cathode buffer is not cloudy. If it is, follow the recipe outlined in **7.2 Buffer Recipe**. Remove the cathode fill cap (Part #017) and leave the cap off whenever possible.

7.4 Discarding Waste

Discard all waste from the cell away from all water sources, especially drinking water. Cell waste may be used for watering plants or crops.

8 MAINTENANCE

8.1 Filter Replacement

The bacterial filter (Part #009) keeps other species of bacteria out of the cell and must be replaced at the beginning of each month. This is done by removing the media storage chamber end cap of the cell (Part #010). Removal of the cap will expose three hoses as well as the filter mechanism. Pull the hose off both sides of the used filter. Insert the new filter into the hose and replace end cap.

8.2 Buffer Replacement

The buffer in the cathode chamber should be replaced monthly, or when it appears very cloudy. Drain the old buffer out of the cathode drain by unscrewing the drain at the bottom of the cathode chamber using the supplied hex key (Part #018). When the chamber is empty replace the cathode drain. Prepare new buffer following the recipe outlined in **7.4 Buffer Recipe**. Fill the cathode chamber with buffer halfway up the neck of the fill cap to the fill line.

9 TROUBLESHOOTING

Problem: The injection pump is very difficult to press or injects no fluid.
Solution: The bacterial filter may be blocked. Replace the filter.

Problem: The injection pump injects only air.
Solution: There may not be enough media in the media storage chamber. Check the level indicator and add media as necessary.

Problem: The MFC does not produce any power.

Solution: There may be a loose connection on one of the terminals. Tighten all connections and retry.

Problem: The MFC still is not producing any power.
Solution: Feed the bacteria 50 pumps of media using the injection pump.

Problem: The media in the media storage chamber is very cloudy.
Solution: It has other species of bacteria growing in it. Dump and refill.

Problem: I ran out of some ingredients.
Solution: Order all supplies from BioVolt online at biovolt.org or over the phone at 1-800-BioVolt.

Problem: Any problems we were unable to answer here?
Solution: Call BioVolt Monday-Friday 8:00AM – 5:00PM (GMT – 05:00) Eastern Time (US & Canada) and one of our trained technicians will assist you.

11 GLOSSARY OF TERMS

ammonium chloride – an ingredient in the media
anode – the negative terminal or side of a battery or fuel cell
bacterial media – the food solution for the bacteria
baking soda – an ingredient in the media

buffer – any solution which resists changes in pH
cathode – the positive terminal or side of a battery or fuel cell
cutaway – a view where some of the structure is removed to reveal previously unseen parts
dibasic sodium phosphate – see sodium phosphate
dissolved – no visible solids remain in a liquid
hex key – a hexagonally shaped piece of metal bent at a right angle. Used for turning screws and bolts.
LED – stands for light emitting diode. A highly efficient form of lighting.
load – the device or object to be powered
media – see ***bacterial media***
media storage chamber – chamber in the MFC-V1 for storing media before it is pumped into the anode
MFC – see ***microbial fuel cell***
MFC-V1 – the microbial fuel cell you own
microbial fuel cell – a type of fuel cell which uses the metabolism of bacteria to create electrical current
non-iodized table salt – an ingredient in the media
sodium phosphate – an ingredient in the buffer solution and media solution.
table salt – see ***non-iodized table salt***
white vinegar – an ingredient in the media